MassMatrix Web Server – Full Manual

Version 2.2.3 or later

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November 2010

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Introduction

Introduction

MassMatrix is a newly developed database search software package for tandem mass spectrometric data. It uses a mass accuracy sensitive probabilistic scoring model to rank peptide and protein matches. MassMatrix provides improvements in sensitivity over Mascot and SEQUEST with comparably low false positives. MassMatrix has additional capabilities that set it apart for other algorithms. It is capable of searching through hierarchical MS^n (n>=3) spectra (useful in phosphopeptide analysis) where higher confidence in peptide ID can be achieved over MS^2 alone. The algorithm is also capable of direct searching of disulfide or chemical cross-linked peptides.

General Features

MS/MS Data: mzXML, MGF, and mzData.
Protein Database: FASTA sequences, MassMatrix BAS format.
Results: Html format.

Quantitation

1) 4,8-plex iTRAQ™
2) 2,6-plex TMT (Thermo Pierce)
3) SILAC and ^{15}N Labeling

Introduction

Unique Aspects

Mass Accuracy Sensitive Probabilistic Scoring Model: Pure statistical model that is sensitive to high mass accuracy.

Generic Searching Algorithms and Models: Isotope labeling, DNA and RNA sequences and carbohydrate side-chain cleavages.
Automated Disulfide Linkage and Chemical Cross-Linkage Searching: Proteins and peptides with disulfide bonds or chemical cross linking can be directly identified without chemical reduction and/or other derivatiization, like normal proteins and peptides.

Hierarchical MS^n (n>=3) spectral data base searching for peptide: MS^2 <=> MS^n (n>=3) search algorithm to raise scores and confidence and search peptides with significant neutral loss such as phosphopeptides. This algorithm can be applied to identify peptides that are difficult to be identified only by MS^2 spectra, such as peptides with multiple phosphorylation sites.

RPLC Retention Time Prediction Model: Retention time is predicted by peptides' hydrophobicity. A statistical score provided based on the model.
Handling Low Quality Spectra: Built-in dynamic noise level (DNL) filtering algorithm to filter noise peaks.

Introduction

Publications

1) Hua Xu, Michael A. Freitas BMC Bioinformatics 2007, 8, 133 (Link)

2) Hua Xu, Michael A. Freitas J. Proteome Res. 2008, 7(7), 2605-2615(Link)

3) Hua Xu, Liwen Zhang, Michael A. Freitas J. Proteome Res. 2008, 7(1), 138-144 (Link)

4) Hua Xu, Lanhao Yang, Michael A. Freitas BMC Bioinformatics 2008, 9, 347 (Link)

5) Hua Xu, Michael A. Freitas Proteomics 2009, 9(6), 1548-1555 (Link)

6) Hua Xu, Liwen Wang, Larry Sallans, Michael A. Freitas Proteomics 2009, 9(7), 1763-1770 (Link)

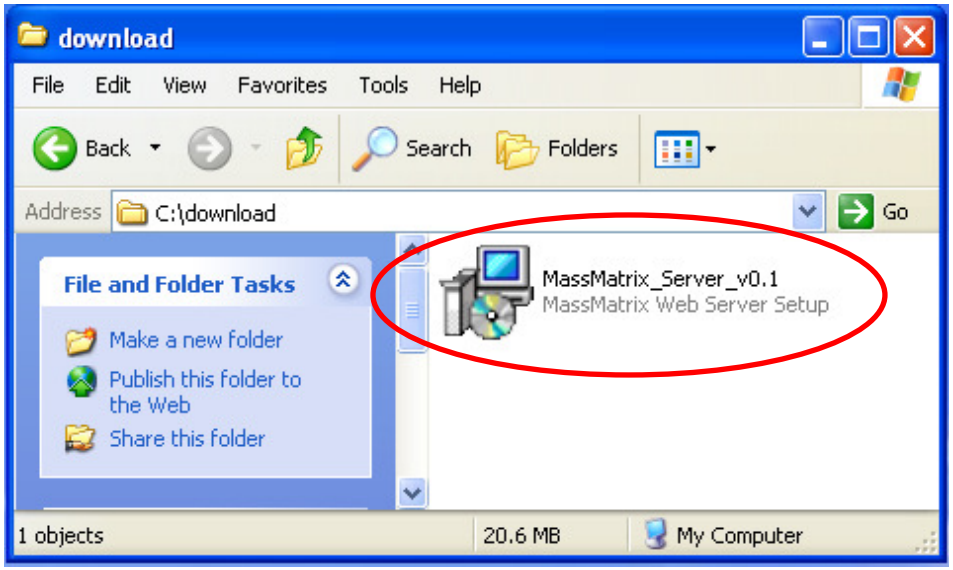
7) Hua Xu, Michael A. Freitas Bioinformatics 2009, 25(10), 1341-1343 (Link)

1. Installation and Uninstallation Manual

1.1 Installation on Windows

Installation on Windows

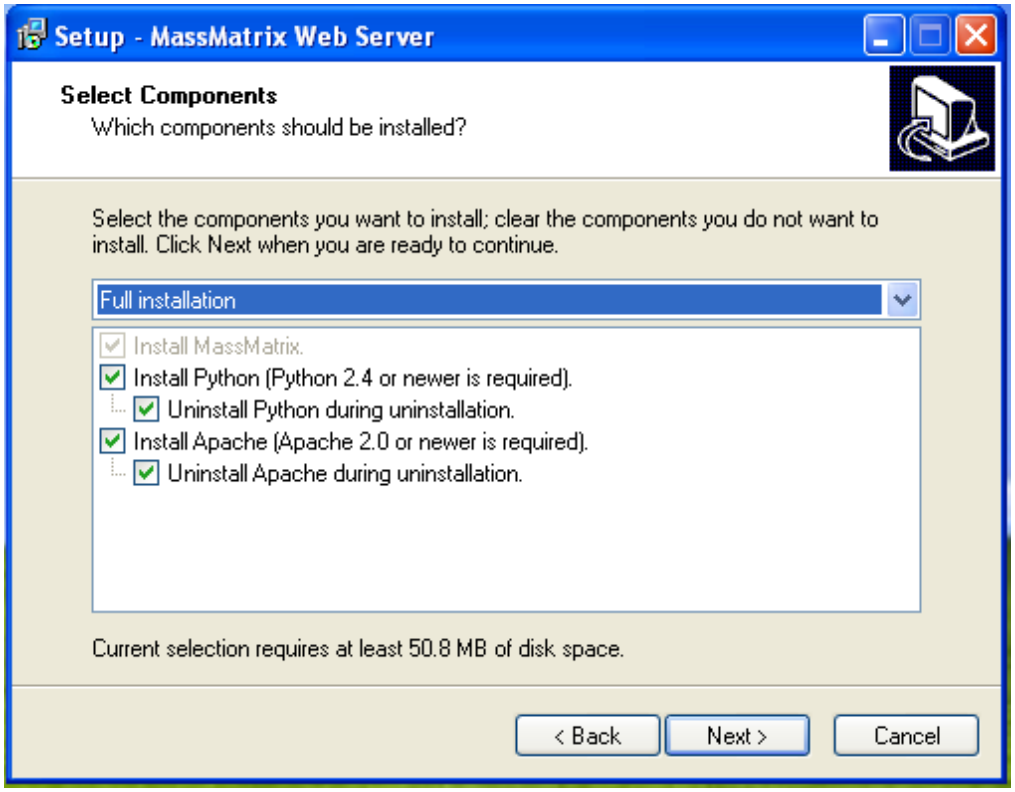
1. **Double click to run the MassMatrix installation file.**



2. **Follow the directions of the installation wizard until you reach the component selection panel.**

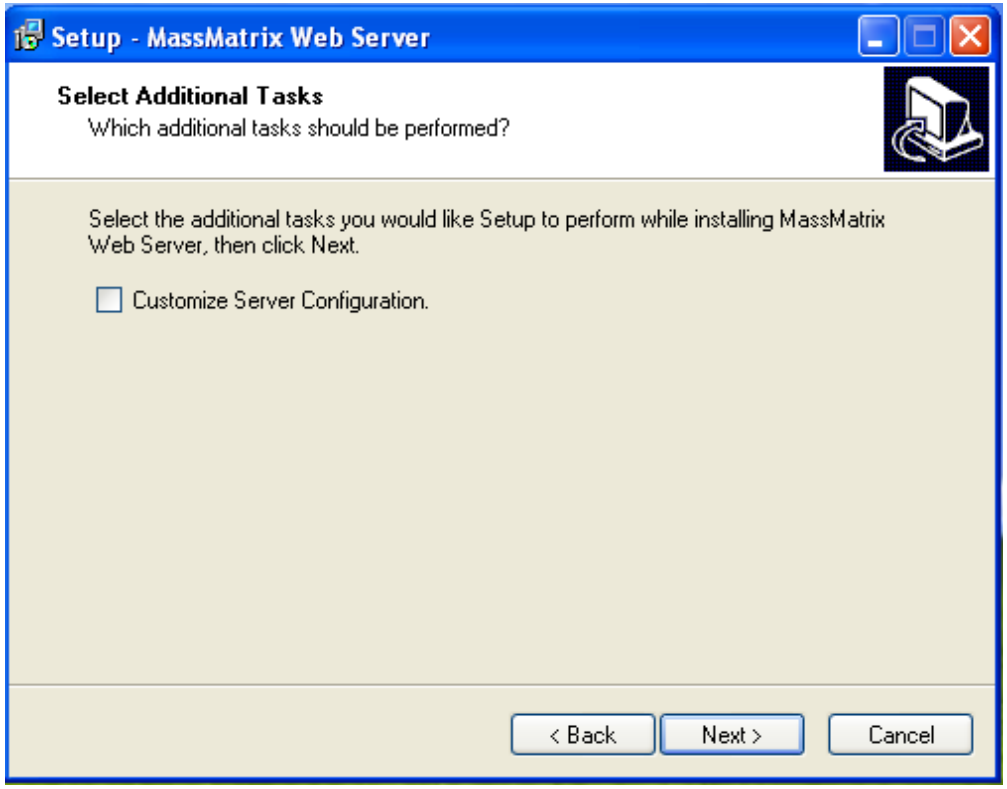
Installation on Windows

3. **Please choose the additionally required packages that you want to install: Python (http://www.python.org/) and Apache 2.0 (http://www.apache.org/). Please unselect the one(s) already installed on your computer. If you don't know, it is very likely that you don't have them. So just choose them all.**



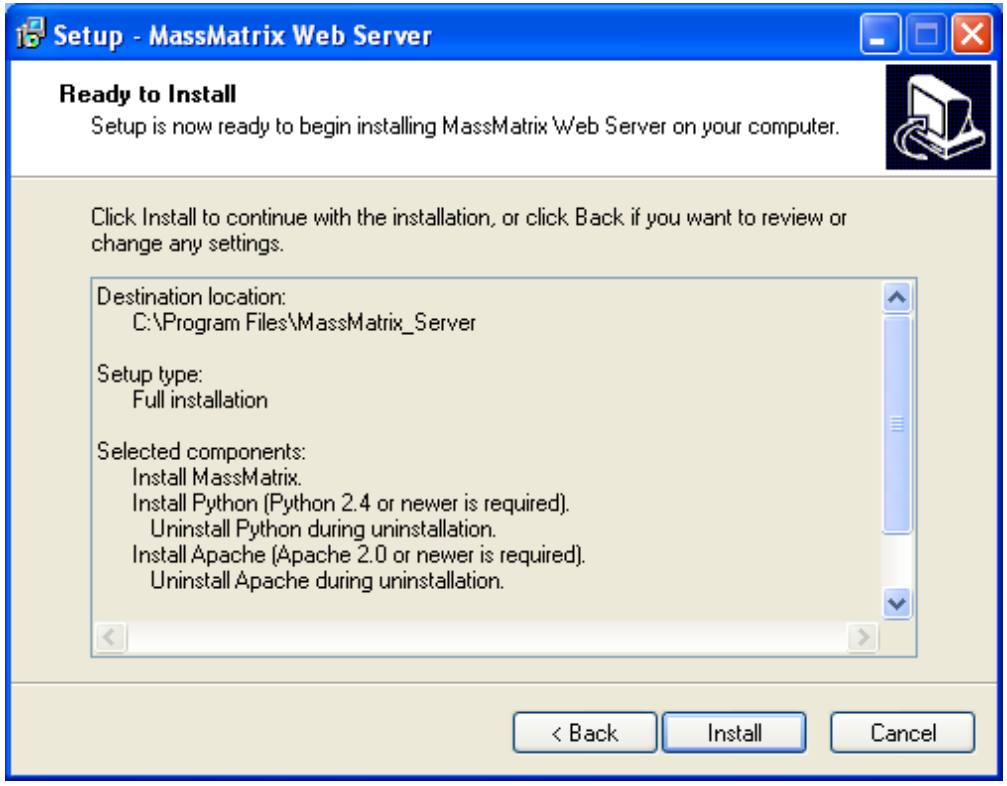
Installation on Windows

4. **Select if you want to customize the MassMatrix server configuration. This is useful when you have multiple servers running on the computer. If you don't have any server running or you don't know anything about http server, please just leave it unselected and the installer will do it for you.**



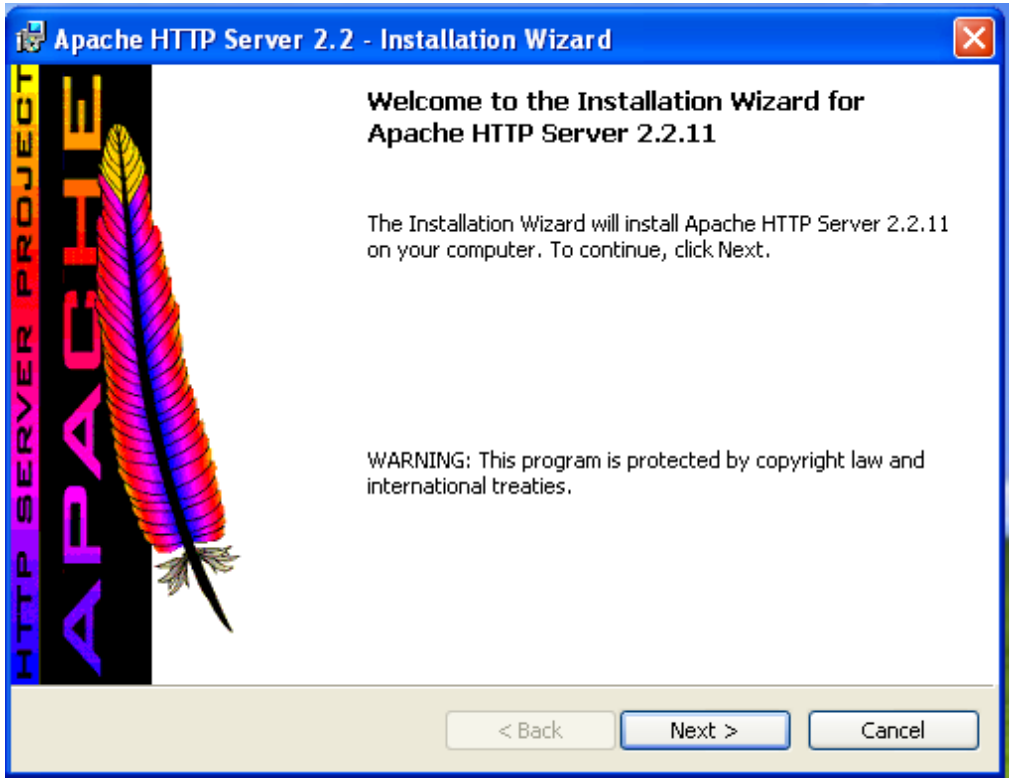
Installation on Windows

5. **Click “Install” to install MassMatrix or “Cancel” to cancel it.**



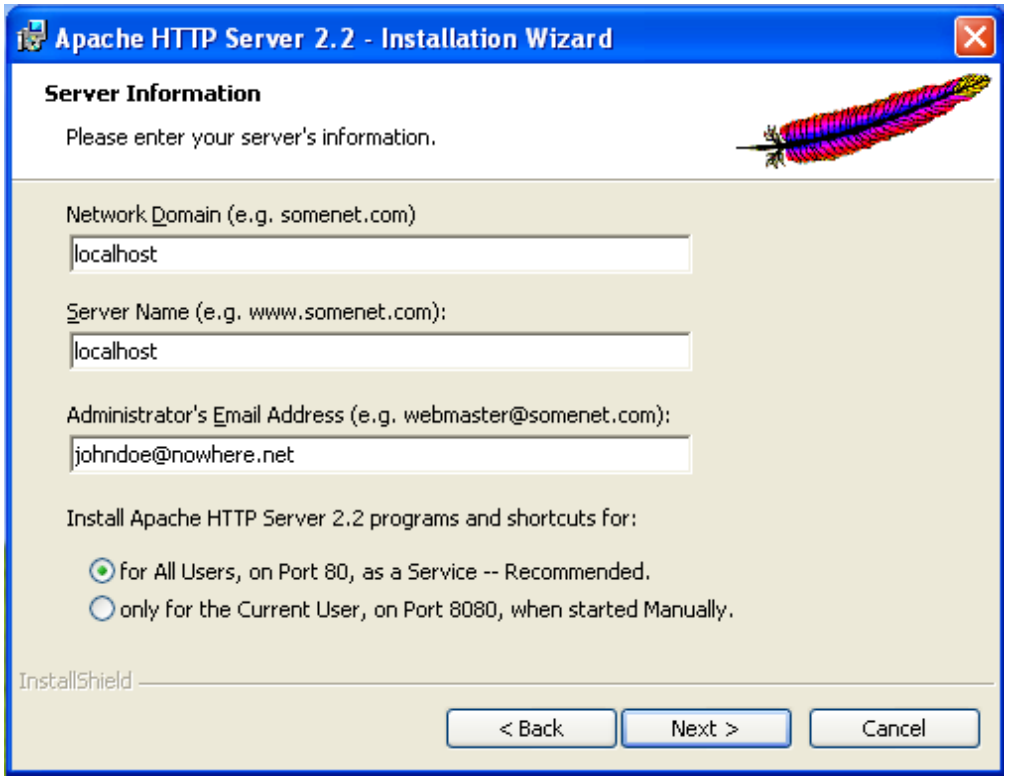
Installation on Windows

6. **If you choose Apache in Step 3, an Apache 2.2 installation wizard will pop up and direct you to install Apache 2.2 (http://www.apache.org/). Otherwise, go to Step 10.**



Installation on Windows

7. **Follow the directions in the Apache installation wizard.**
8. **Please type "*localhost*" in the *Network Domain* field, "*localhost*" in the *server name* field unless this server will be public and you know what its domain and server name are. Provide an email address (it doesn't have to be real) in the filed of admin's email.**



9. **Follow the directions to install Apache 2.2.**

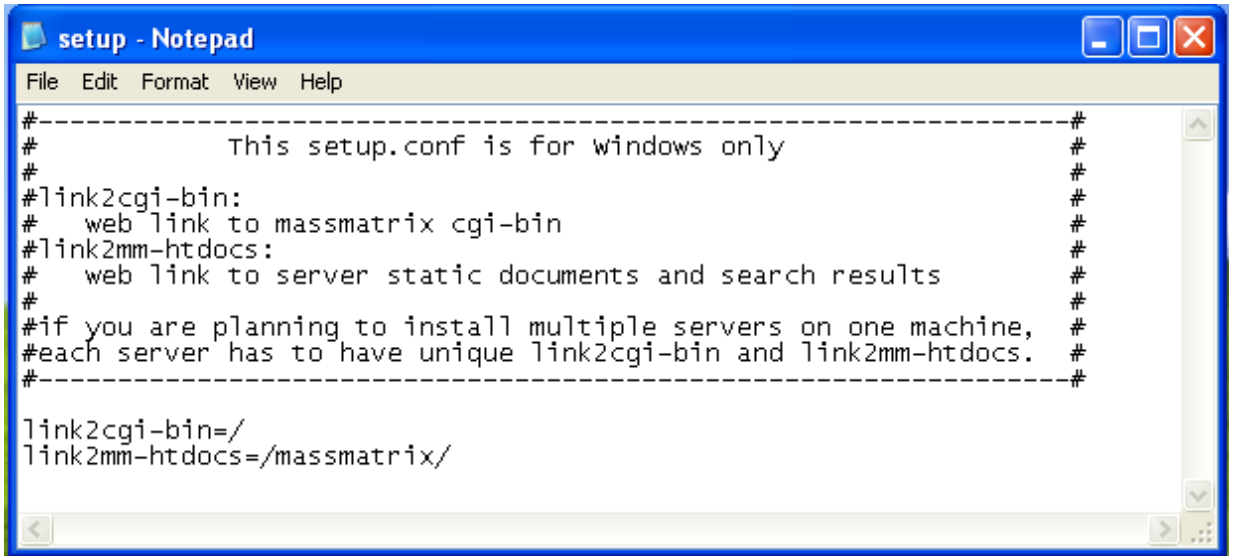
Installation on Windows

10. If you choose Python in Step 3, a Python installation wizard will pop up and direct you to install Python 2.5 (http://www.python.org/). Otherwise please go to Step 12.

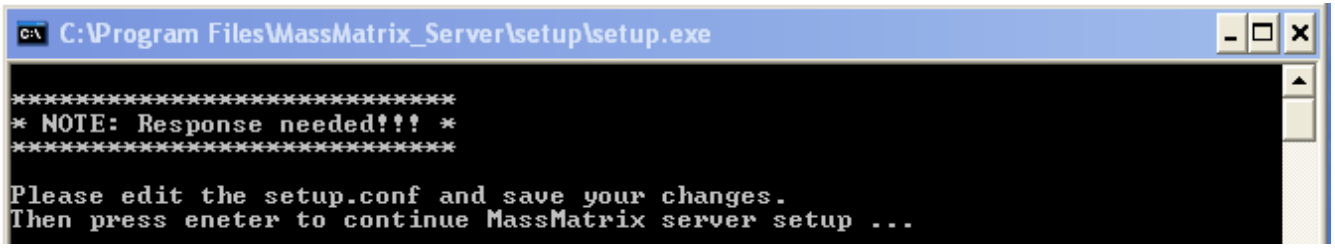
11. Follow the directions to finish installing Python.

Installation on Windows

12. A DOS window will be prompted. If you choose to customize the MassMatrix server configuration in Step 4, the server configuration file will be opened in Notepad for you to edit. Please edit, save and close the configuration file. Otherwise please go to step 14.

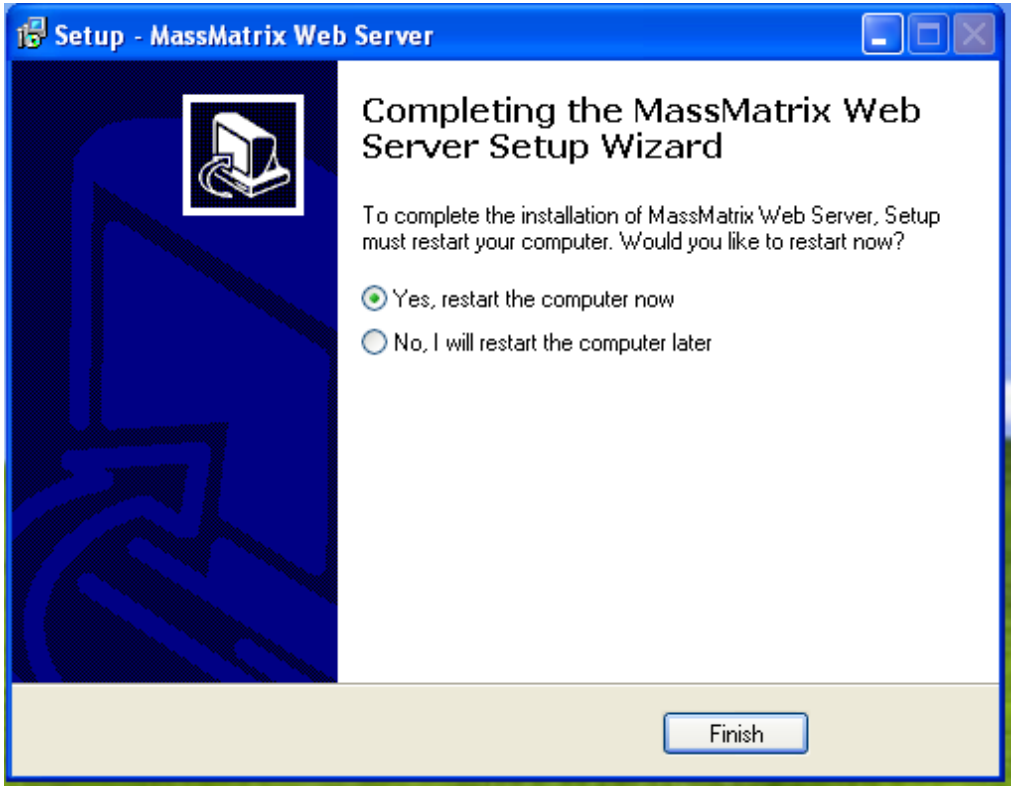


13. Press “Enter” in the prompted DOS window to continue.



Installation on Windows

14. **Click “Finish” to finish the installation process. You will have to restart the computer after installation in order to make MassMatrix web server work properly. Please restart your computer.**

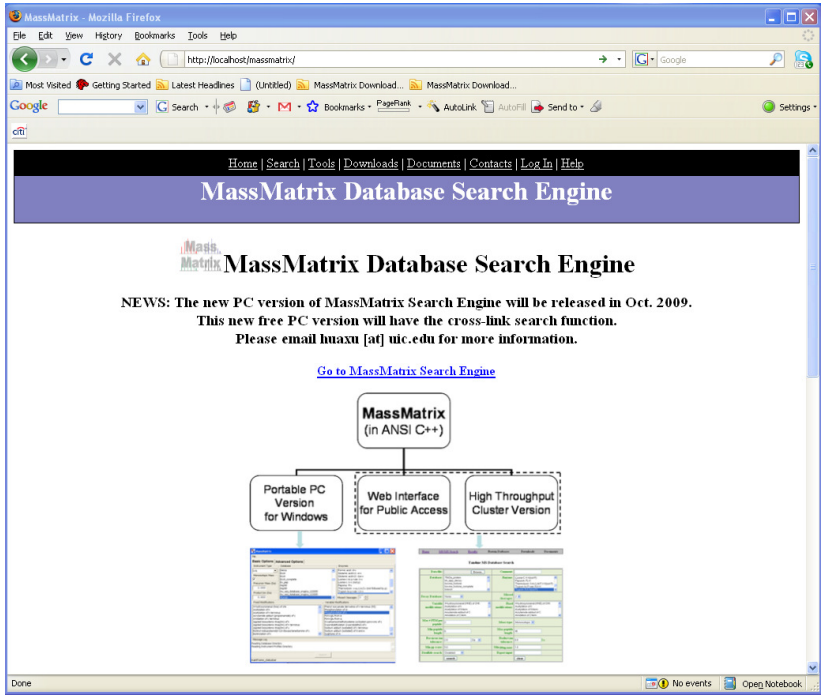


15. **After restart of you computer, the MassMatrix search monitor and Apache are automatically running on the taskbar.**

Installation on Windows

16. **The MassMatrix web server is successfully installed and ready to use at (unless you changed link2mm-htdocs at Step 12):**

http://localhost/massmatrix/ (locally)
http://IP-of-your-server/massmatrix/ (remotely)*



* In order to make your MassMatrix server remotely accessible on other computers, you will have to open the port (80 by default if you didn't change it during Apache 2.2 installation) for the server in the firewall settings.

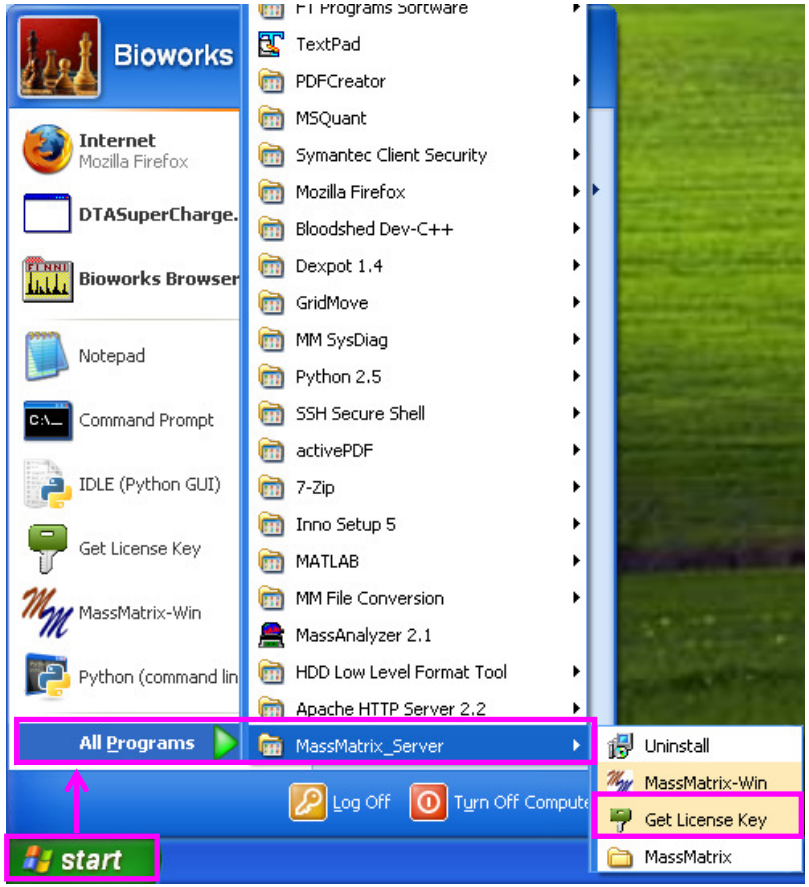
To do that, you may open the Network Connections folder through **Start -> Control Panel -> Network Connections**. On the left pane, click on 'Change windows firewall settings' under Network tasks. Flip to the 'Exceptions' tab and click on 'Add Port…" to add the port.

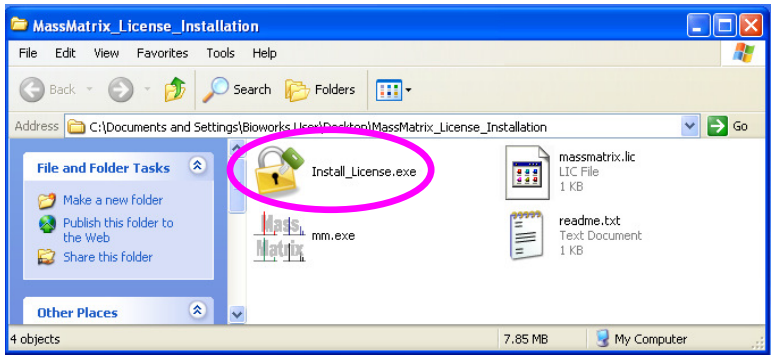
17. **Please log in as admin to change the admin password and set up the server by referring to the administrator manual at:**

https://sourceforge.net/projects/massmatrix/files/MassMatrix_Manuals/MassMatrix%20Web%20Server-Admin%20Manual.pdf/download

Request and Installation of MassMatrix License

1. Please request a license for your server through "**start -> All Programs -> MassMatrix Server -> Get License Key**". (You may do that through the web server too.)

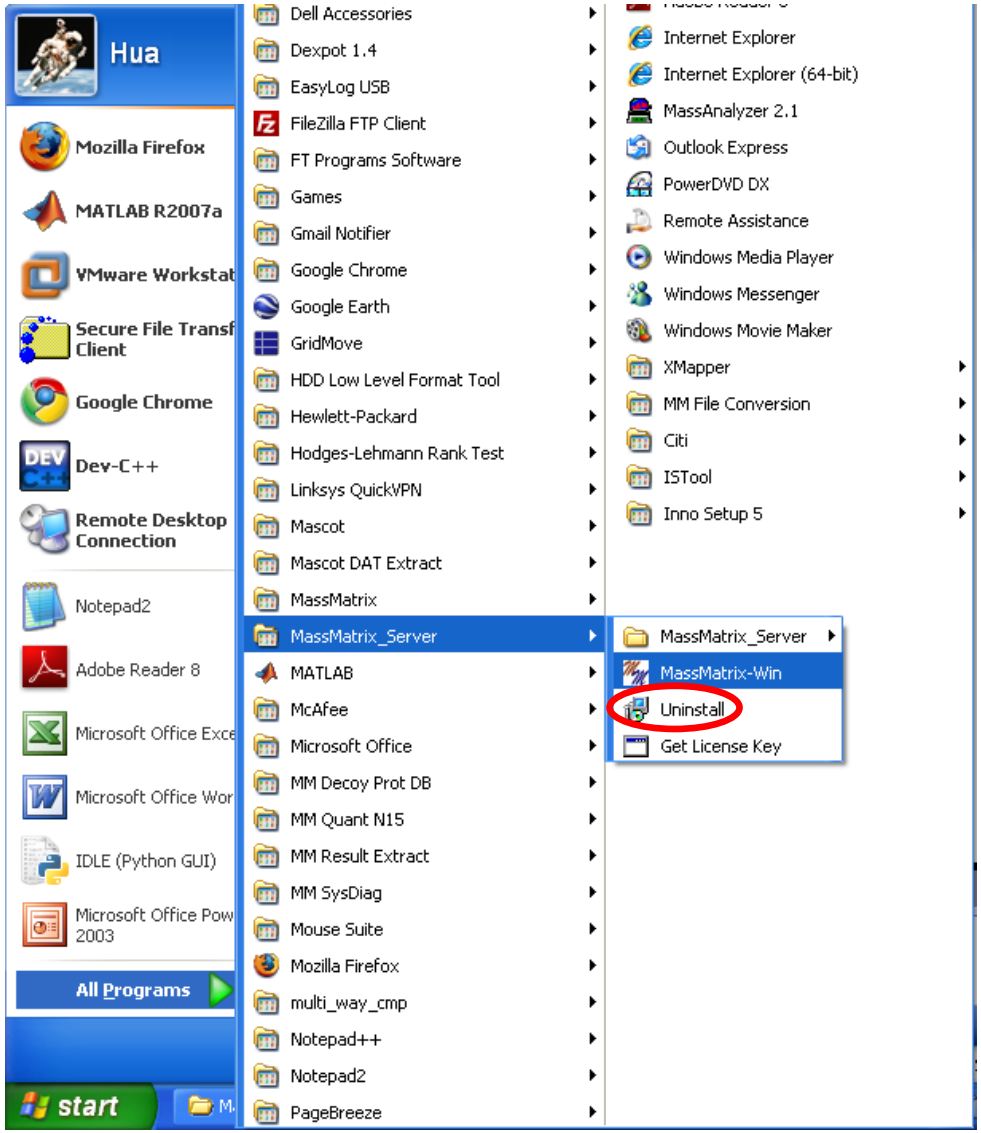




2. After receiving your license installation, please run "Install_License.exe" in the package to install a license.

Uninstallation on Windows

Uninstall MassMatrix though
“start -> All Programs -> MassMatrix Server -> Uninstall”.



1.2 Installation on Linux

Installation on Linux

Prerequisites for installing MassMatrix server on Linux

1. Python 2.4 or newer installed

2. Apache2 or other HTTP server installed and running

3. GD library installed.

Note: Python, Apache HTTP server and GD library normally come with your Linux distribution. Most of Linux distributions, such as Red Hat, Fedora, and SUSE, have Python and Apache. So you don't have to worry about these prerequisites.

Installation on Linux

Installing MassMatrix server on Linux

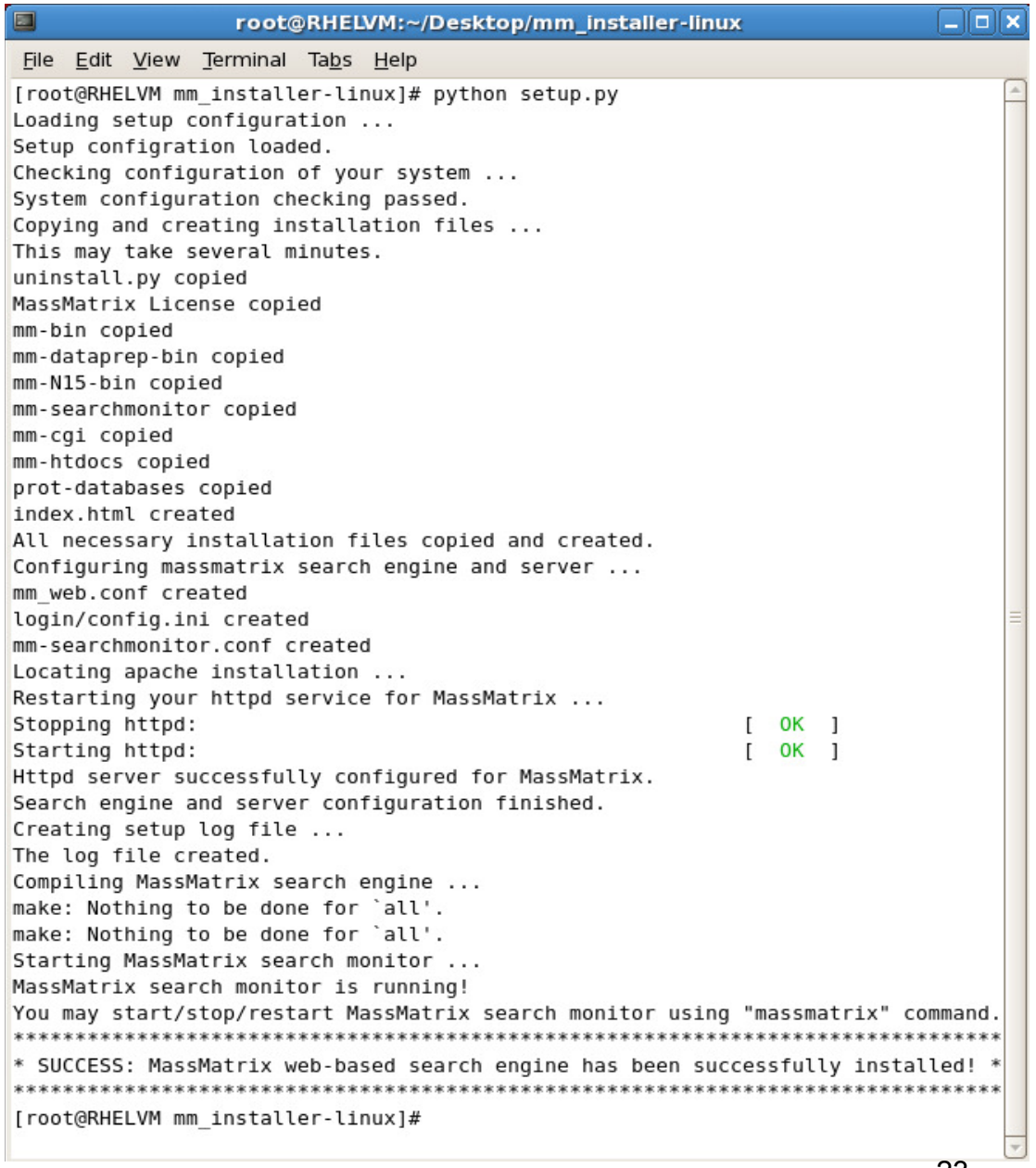
1. Unzip the installation package and cd to that directory.

   $ cd {path to installation dir}

2. Edit the setup.conf by referring to the instructions in the file.

3. Install MassMatrix.

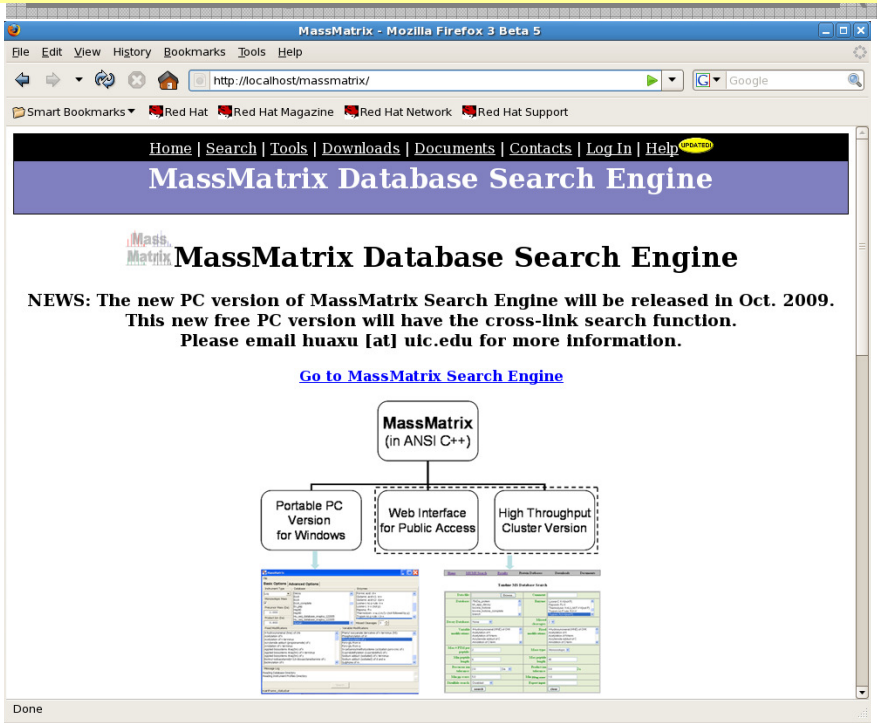
   $ python setup.py



Installation on Linux

4. The MassMatrix web server is successfully installed and ready to use at (unless you changed link2mm-htdocs in setup.conf):

   http://localhost/massmatrix/ (locally)
   http://IP-of-your-server/massmatrix/ (remotely)



5. Please log in as admin to change the admin password and set up the server by referring to the administrator manual at:

https://sourceforge.net/projects/massmatrix/files/MassMatrix_Manuals/MassMatrix%20Web%20Server-Admin%20Manual.pdf/download

Uninstallation on Linux

1. cd to the installation directory

   $ cd {installation directory}

2. Uninstall MassMatrix on Linux

   $ python uninstall.py

1.3 Installation on Linux Cluster

Installation on Linux Cluster

Prerequisites for installing MassMatrix server on a Linux cluster

1. Python (***2.5 or newer***) and Apache (or other HTTP server) need to installed on the head node. GD library and SSH server need to be installed on the head node and all slave nodes.

2. MassMatrix should be installed on the head. Nothing needs to be installed on the slaves.

3. The installation directory on the head needs to be mapped on all slaves. For example, "/share/mm/" on the head node is the installation dir, It should also be mapped and accessible on all slaves as "/share/mm".

Note: In order to set up MassMatrix on a cluster, you need to have advanced knowledge in Linux to set up a shared hard drive on cluster and start SSH servers on all slaves. Otherwise, please consultant with some local experts in Linux.

Installation on Linux Cluster

Installing MassMatrix server on a Linux Cluster

1. Unzip the installation package and cd to that directory.

   $ cd {path to installation dir}

2. Edit the setup.conf by referring to the instructions in the file.

3. Install MassMatrix.

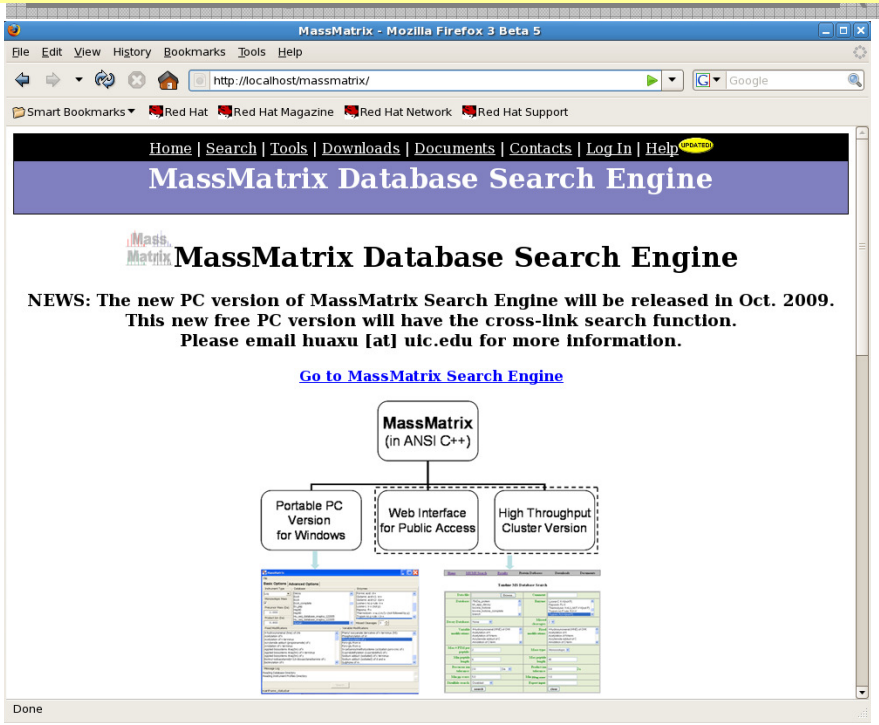
   $ python setup.py --for-cluster

4. Read any warning and error messages during installation and take proper actions according to the given instructions. If you have questions or ecounter problems, please email hxx58@case.edu for help and support.

Installation on Linux Cluster

5. The MassMatrix web server is successfully installed and ready to use at (unless you changed link2mm-htdocs in setup.conf):

http://localhost/massmatrix/ (locally)
http://IP-of-your-server/massmatrix/ (remotely)



6. Please log in as admin to change the admin password, setting up the server and adding slave nodes to the server by referring to the administrator manual at:

https://sourceforge.net/projects/massmatrix/files/MassMatrix_Manuals/MassMatrix%20Web%20Server-Admin%20Manual.pdf/download

Uninstallation on Linux Cluster

1. cd to the installation directory

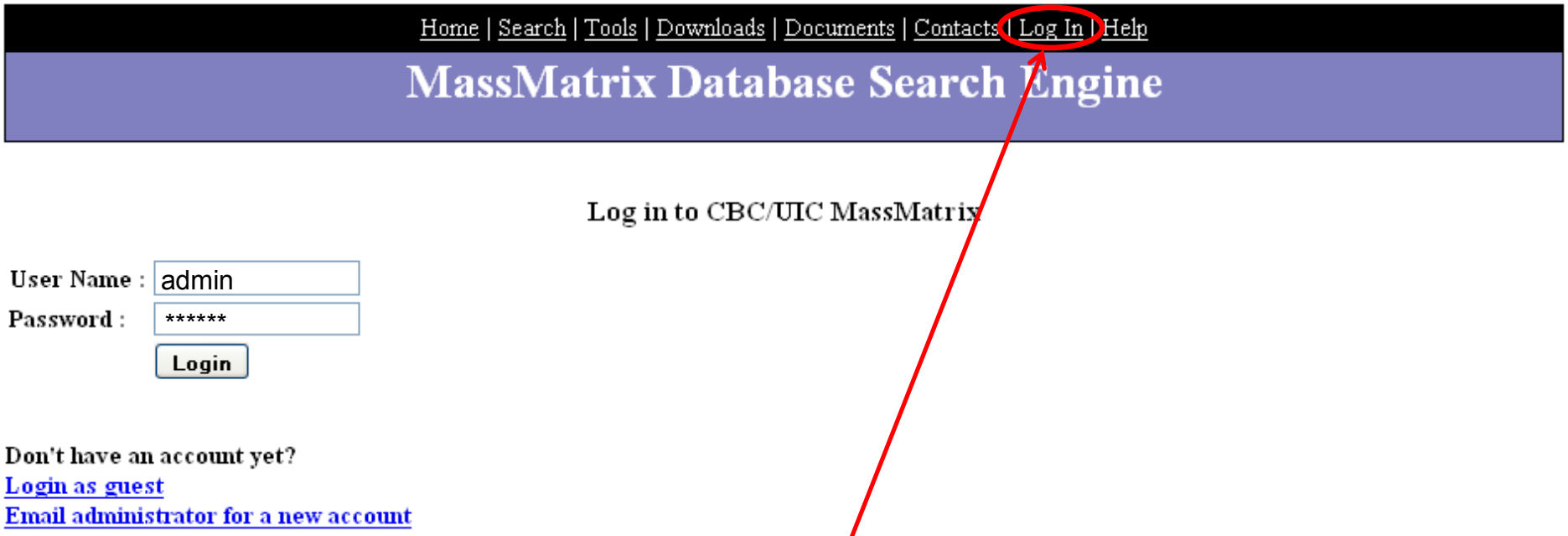
   $ cd {installation directory}

2. Uninstall MassMatrix on Linux

   $ python uninstall.py

2. Administrator Manual

MassMatrix Web Server – Administration

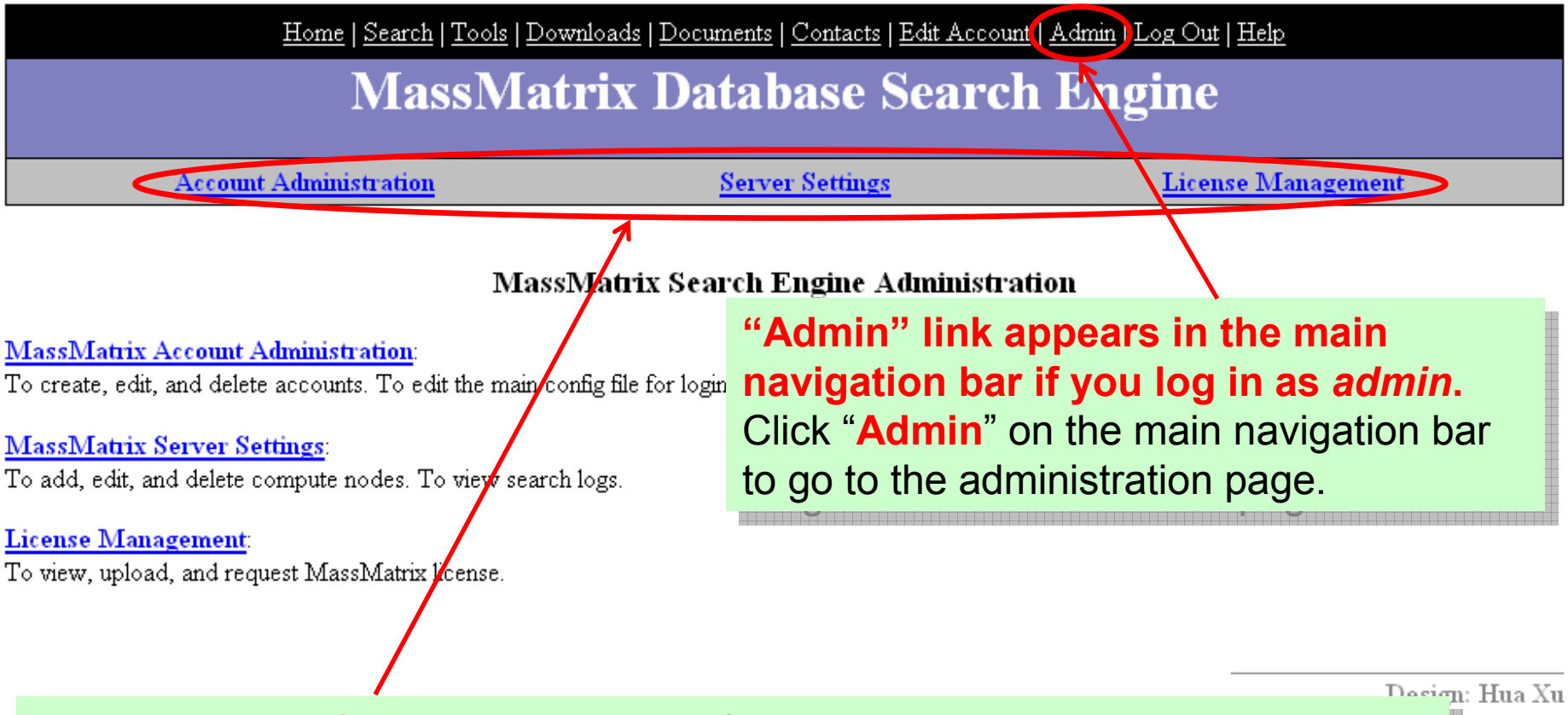


The amin is the only administrator account of the server. It is automatically created during installation. The initial password for admin is “mm1234”. Click “Log In” to log in as admin.

MassMatrix Web Server – Administration

Click "**Edit Account**" on the main navigation bar to go to the account editing page to change the admin password. It is extremely important for you to change the admin password.

MassMatrix Web Server – Administration



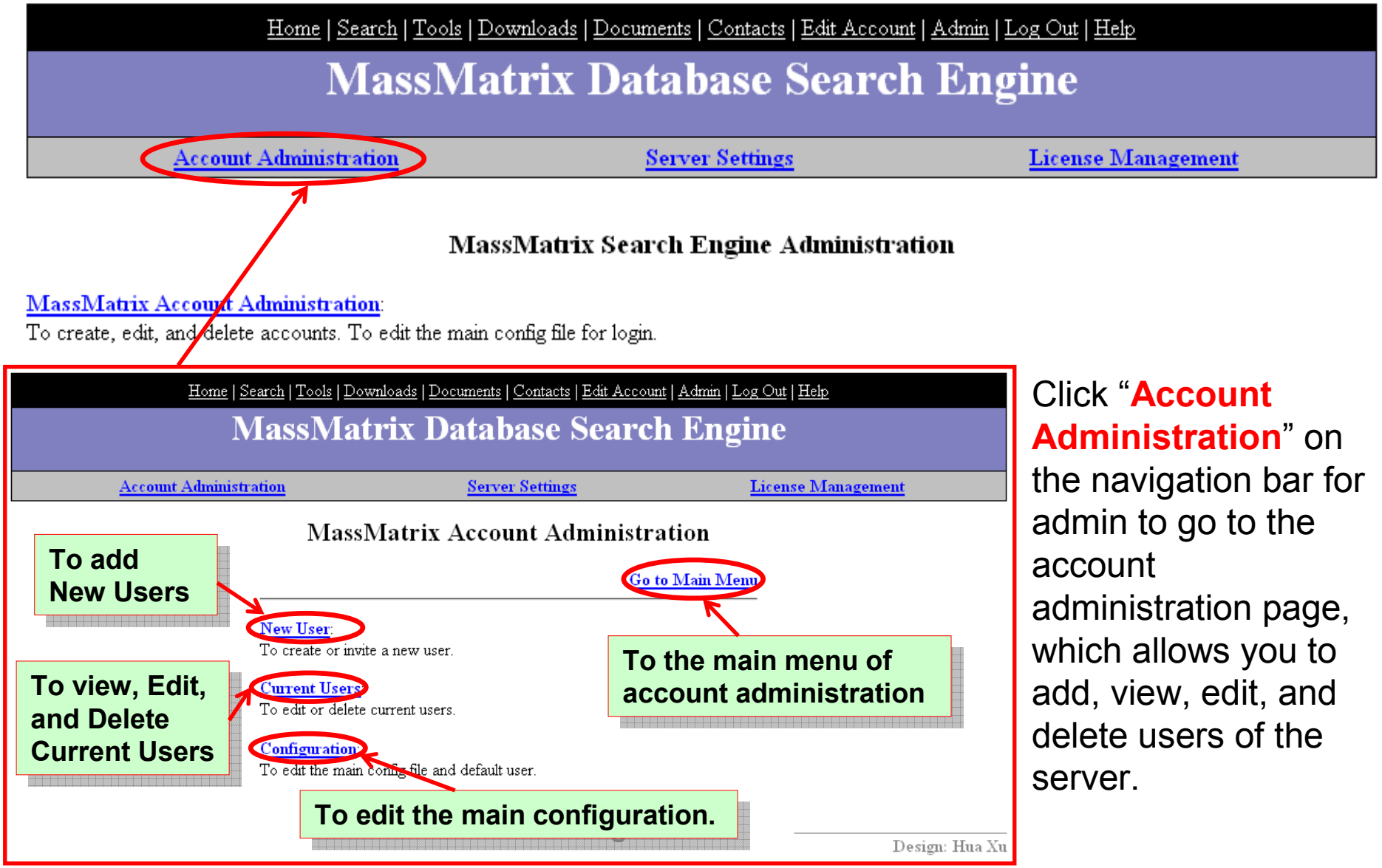
"Admin" link appears in the main navigation bar if you log in as *admin*. Click "**Admin**" on the main navigation bar to go to the administration page.

Navigation Bar for Admin appears after you go to the administration page.

There are three types of administration:

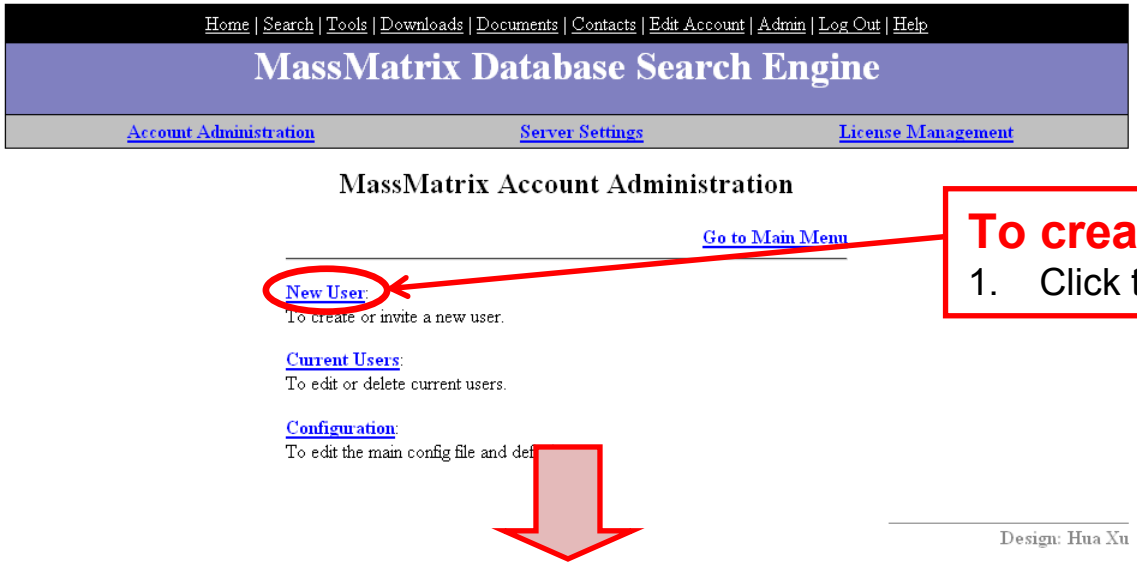
1) Account administration
2) Server settings
3) License management

MassMatrix Web Server – Account Administration



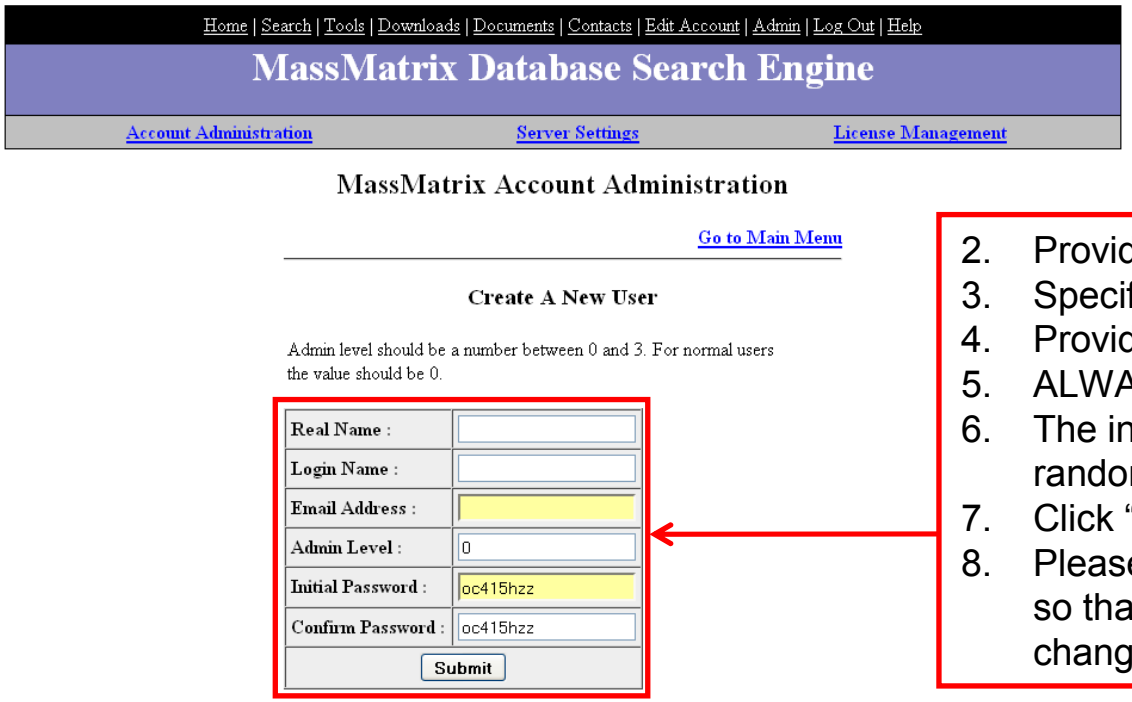
Click "**Account Administration**" on the navigation bar for admin to go to the account administration page, which allows you to add, view, edit, and delete users of the server.

Account Administration – Create A New Account



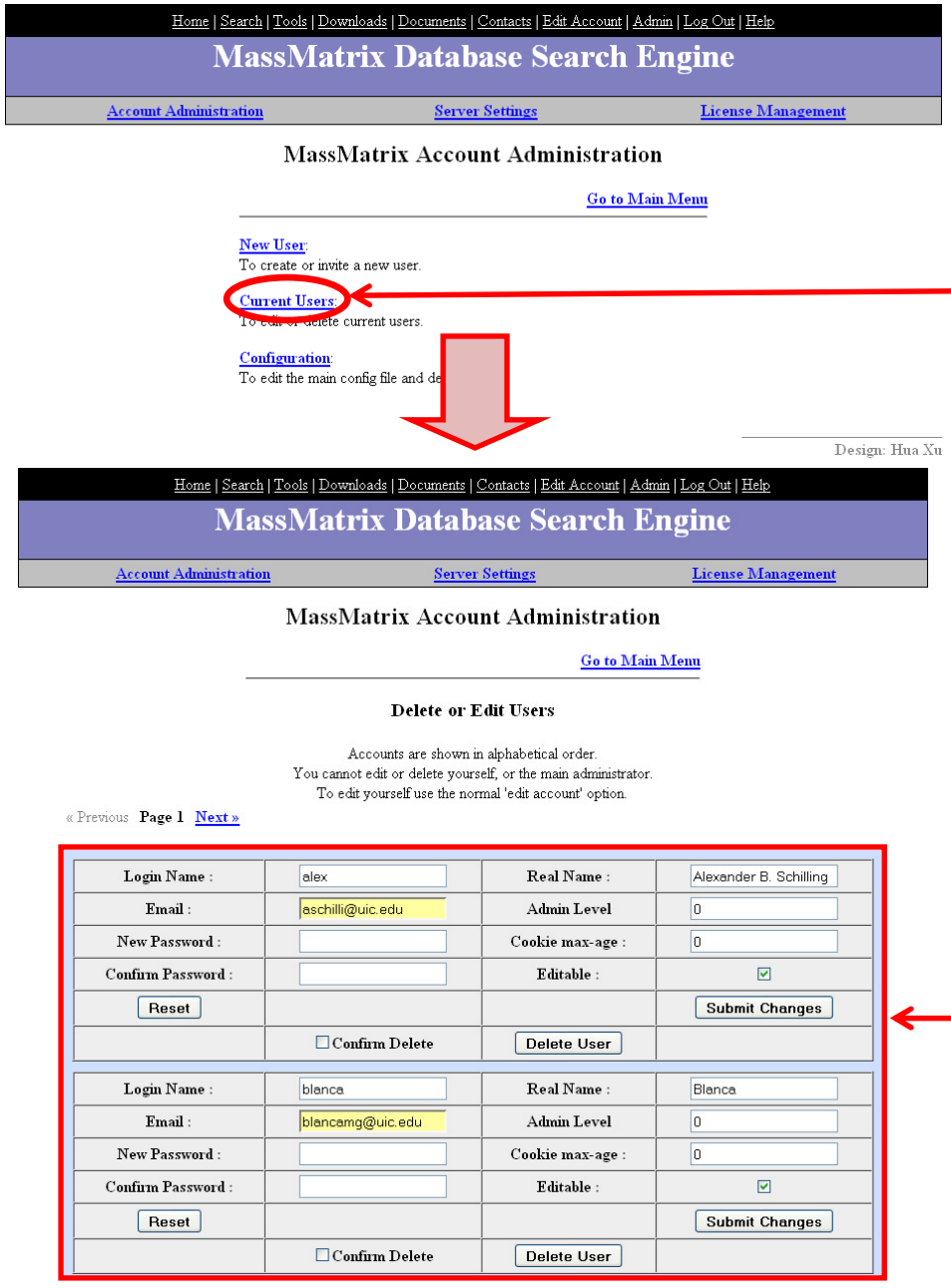
To create a new account:

1. Click the “New User” link.



2. Provide the real name of the user.
3. Specify the username.
4. Provide the email address of the user.
5. ALWAYS leave the admin level be 0.
6. The initial password is automatically and randomly generated. You may change it.
7. Click “Submit” button to create the new user.
8. Please provide this initial password to the user so that you can log in the server. They can change it after logging in.

Account Administration – Edit Existing Accounts



To view, edit and delete existing accounts:

1. Click the "Current Users" link.

Two user accounts are automatically created during installation: amin and guest. The amin will not be shown in the user list and cannot be deleted. The guest user is reserved for guest login for the server. The password for the guest account doesn't matter and can be anything.

The user accounts are shown in pages. Each page has up to 5 users. You can go through all pages using the links of "Previous" and "Next". You may delete users one by one. You may edit user accounts.

Note: If you delete the reserved "guest" account, no guest login will be available for the server. You man create the guest account after you delete it.

Account Administration – Configuration

MassMatrix Account Administration

Go to Main Menu

New User:
To create or invite a new user.

Current Users:
To edit or delete current users.

Configuration:
To edit the main config file and default user.

Design: Hua Xu

To edit the main configuration:

1. Click the "Configuration" link.

MassMatrix Account Administration

Go to Main Menu

From this screen you can edit a few of the settings in the main config file. You can also change some of the settings for the *default user*. These values are transferred to every new account.

Config File Values

This is a description of all the config file values that you can edit from this page.

adminmail
This is the email address that the new account reqeusts will be sent to. Please change it to the email address of the administrator of this server.

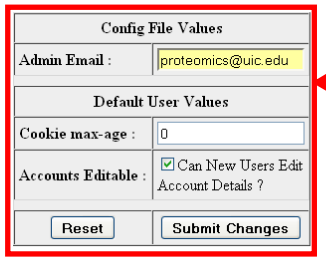
Default User Values

This is a description of all the default user values that you can edit from this page.

max-age
This is the maximum age of the cookie we use, in seconds. Setting it to zero usually means (slightly browser dependent) the cookie will only endure for that browser session. Common values are 3600=1 hour, 86400=1 day, 604800=1 week. The cookie is reset after every new page access - so this is the maximum time in between visits that the cookie will last. After that, the user will have to login again.

editable
This is whether the user is allowed to change their password, email address etc.

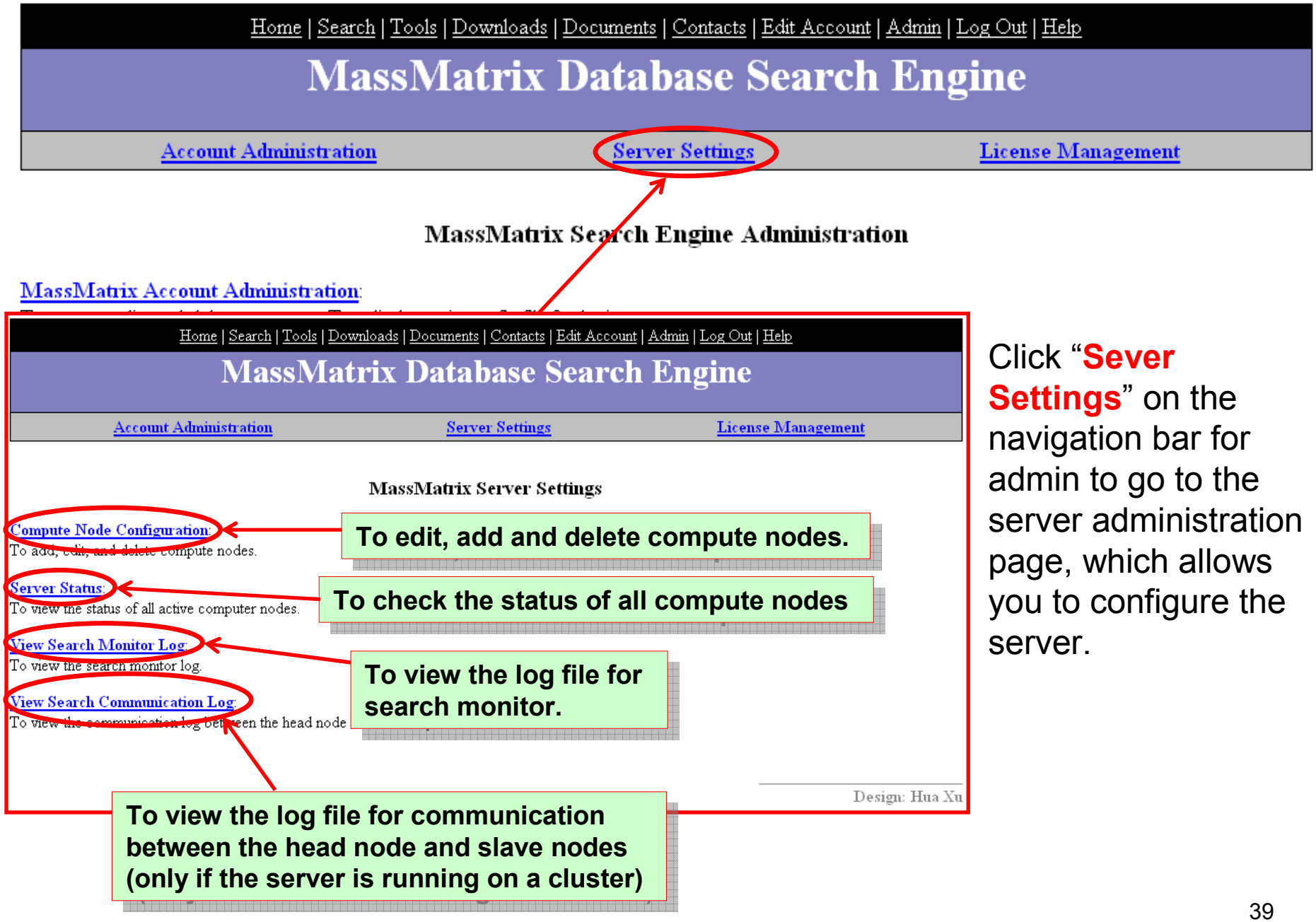
Edit the Values



Please read the instruction on the page to make changes to the main config file of account administration.

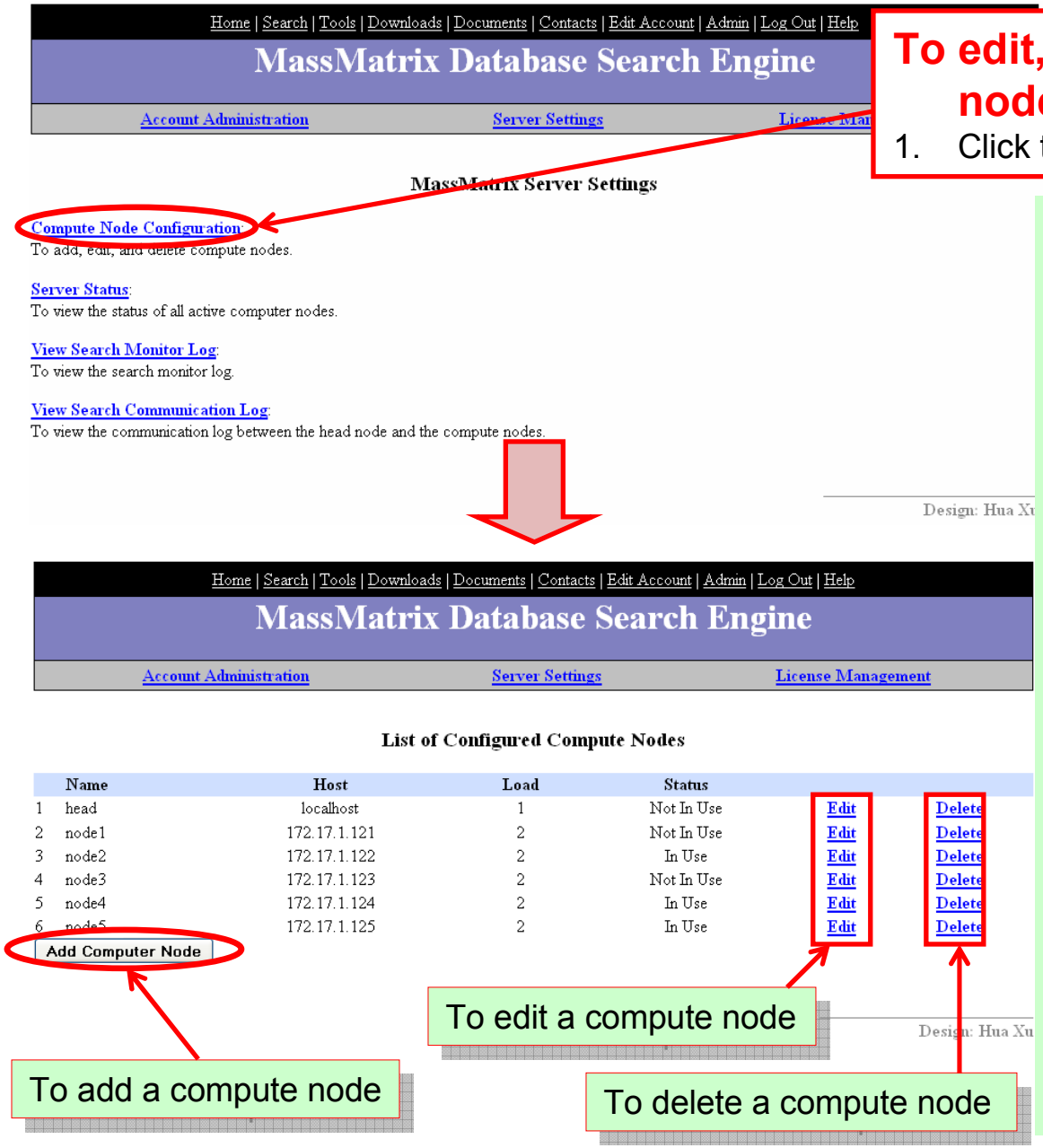
Please make sure that you change the "Admin Email" to the email address of the "real" administrator of the server. New account requests will be sent to this email address.

MassMatrix Web Server – Sever Administration



Click "**Sever Settings**" on the navigation bar for admin to go to the server administration page, which allows you to configure the server.

Sever Administration – Compute Node Configuration



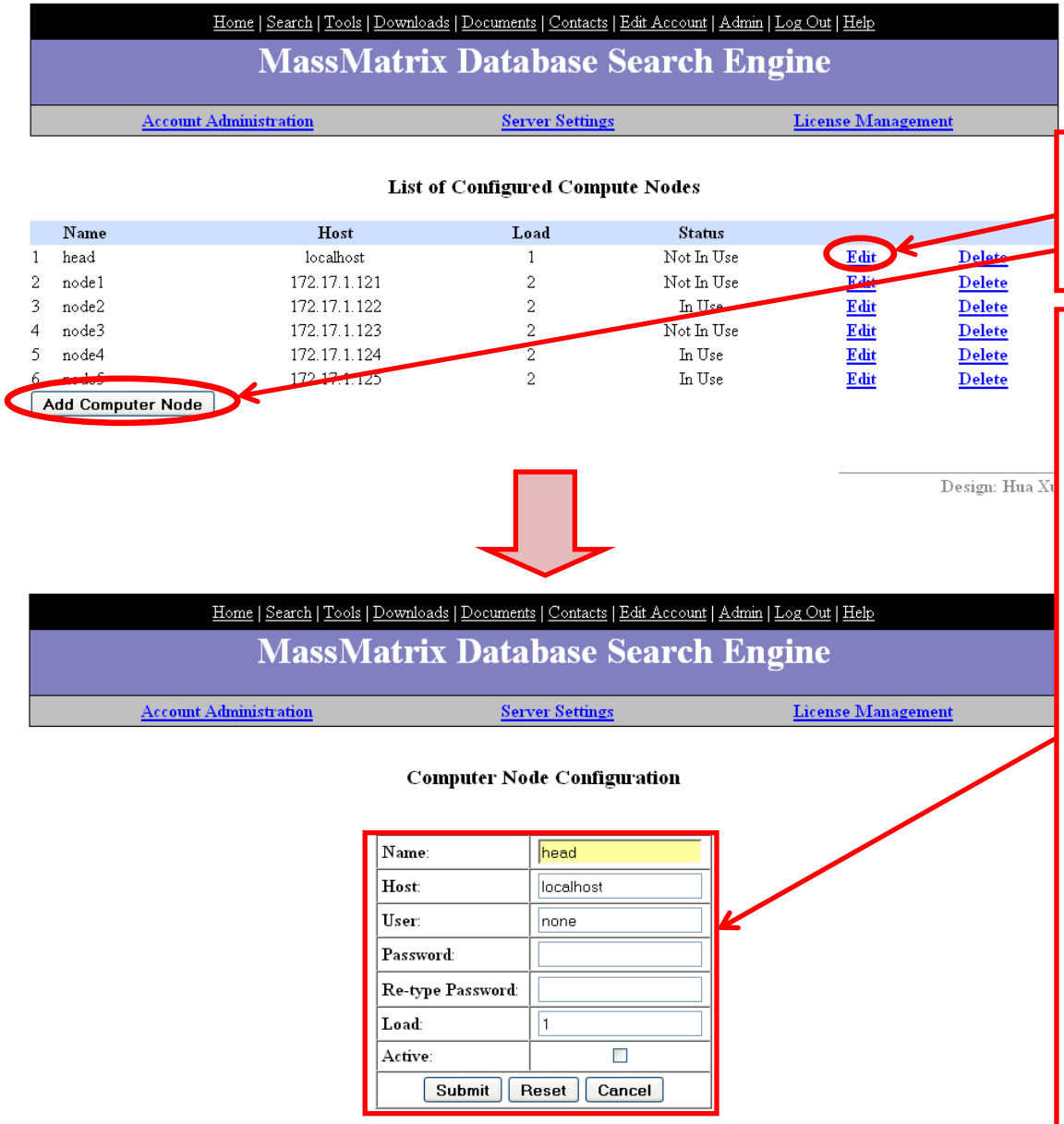
To edit, add and delete compute nodes:

1. Click the "Compute Node Configuration" link.

MassMatrix web-based search engine is for both PC and Linux cluster. If it is running on a PC, you should only have a node called head (your PC) for database search. If it is running on a cluster, you may have many computer nodes (one of them is head where you installed MassMatrix).

For a compute node, its load is the maximum number of search jobs that can be run on the node at the same time. If you have multi-core CPU or multi-CPU on the node, you can specify the load be > 1. Its status is whether the node is in use or not. If the node is disabled, its status is "Not In Use". For a cluster, you may want to disable the head node so that the head node will not do any searches. This can keep the head node from crashing. If other nodes except head node crash, the server can still be running as long as there is one compute node in use is still alive.

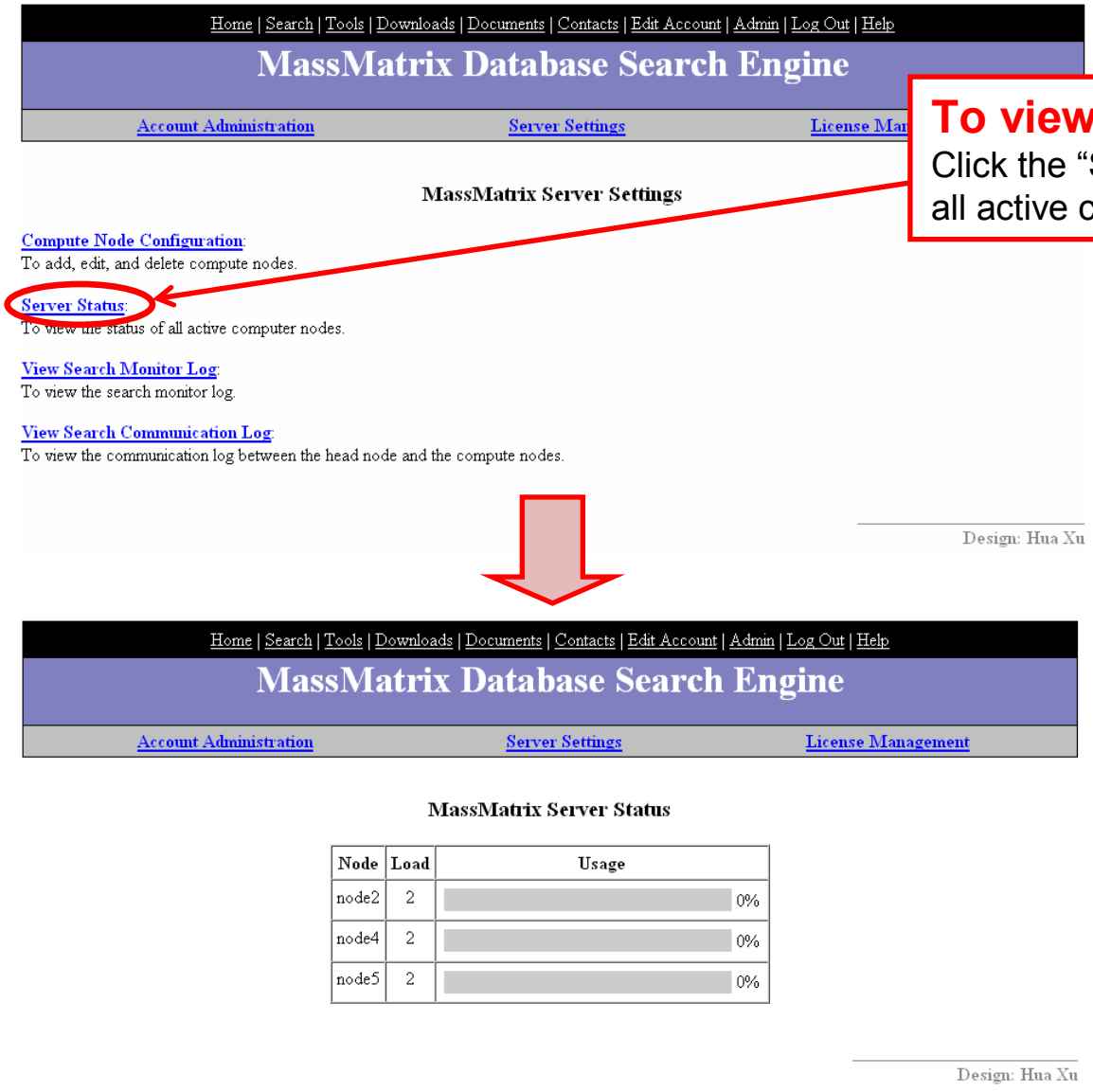
Sever Administration – Compute Node Configuration



To add or edit a node:

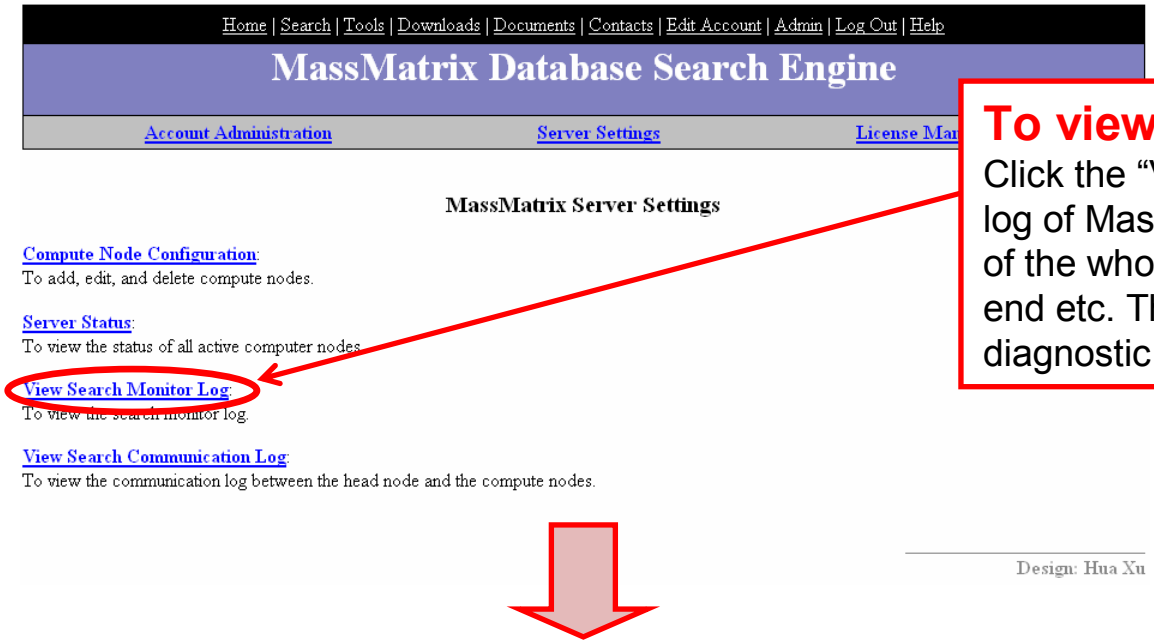
1. Click the "Add Computer Node" icon or the "Edit" link of a compute node.
2. Provide the name of the computer. The name is for your reference only.
3. Specify the *IP address* of the computer node if it is remote or *localhost* if it is the local head node where you installed MassMatrix.
4. Provide the *user name* used to ssh to the computer node if it is remote. Otherwise just put *none* for the local head node.
5. Provide the password used to ssh to the remote node or leave it blank for the local head node.
6. ***Specify the load of the node, i.e. the maximum number of search jobs that can be run on the node at one time.***
7. Specify whether the node is active or not. If the node is not active, it will not be used by the MassMatrix server.
8. Click "Submit".

Sever Administration – Server Status



To view the status of the server:
Click the "Server Status" link to check the status of all active compute nodes.

Sever Administration – Search Monitor Log

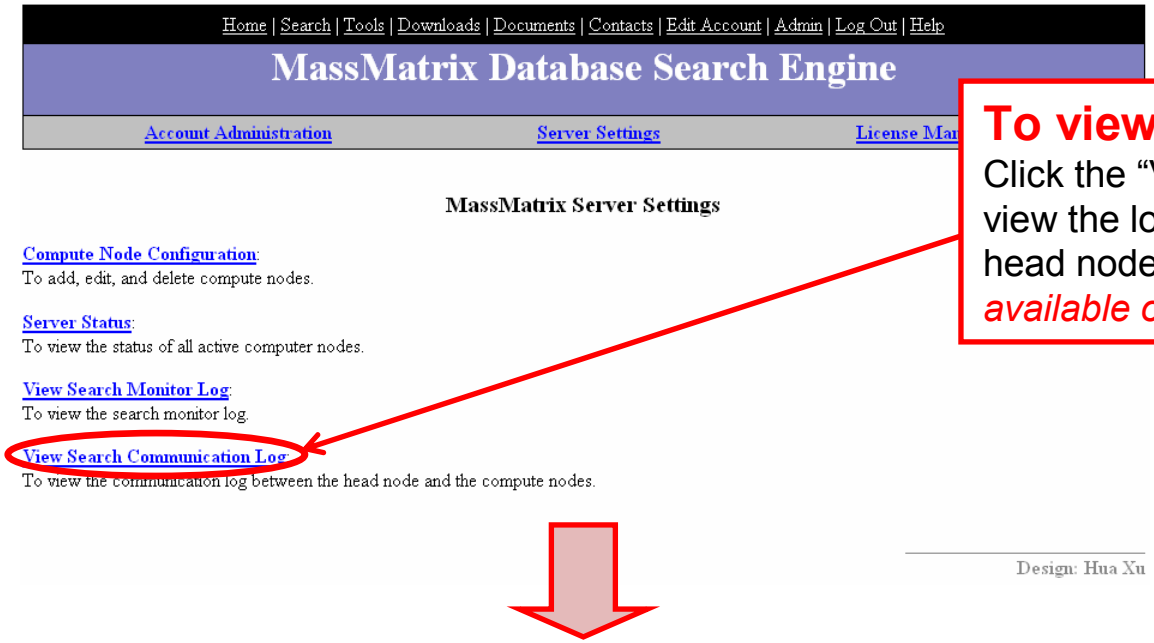


To view the search monitor log:
Click the "View Search Monitor Log" link to view the log of MassMatrix search monitor. It contains the log of the whole sever, including search jobs, error log end etc. This log information is valuable for the diagnostic of the search monitor when it fails.

mm-searchmonitor.log

```
#==========================================================================#
#    MassMatrix Search Monitor                                             #
#    Copyright (C) 2008 Hua Xu, University of Illinois at Chicago          #
#    Contact: huaxu@uic.edu                                                #
#    This software is provided free                                        #
#    Redistribution and modification of the program are prohibited         #
#      without written permission from Hua Xu at huaxu@uic.edu             #
#                                                                          #
#    This program is distributed WITHOUT ANY WARRANTY                      #
#==========================================================================#
******************************************************
* mm-searchmonitor started on Fri Jun 12 13:36:53 2009 *
******************************************************
[Fri Jun 12 13:38:08 2009] The search for job 2262 has been submitted to node1
[Fri Jun 12 13:38:09 2009] The search for job 2264 has been submitted to node1
[Fri Jun 12 13:39:26 2009] Final status of job 2262: Success
[Fri Jun 12 13:39:32 2009] Final status of job 2264: Success
#NOTE# mm-searchmonitor is idle on Fri Jun 12 13:39:32 2009
```

Sever Administration – Search Communication Log

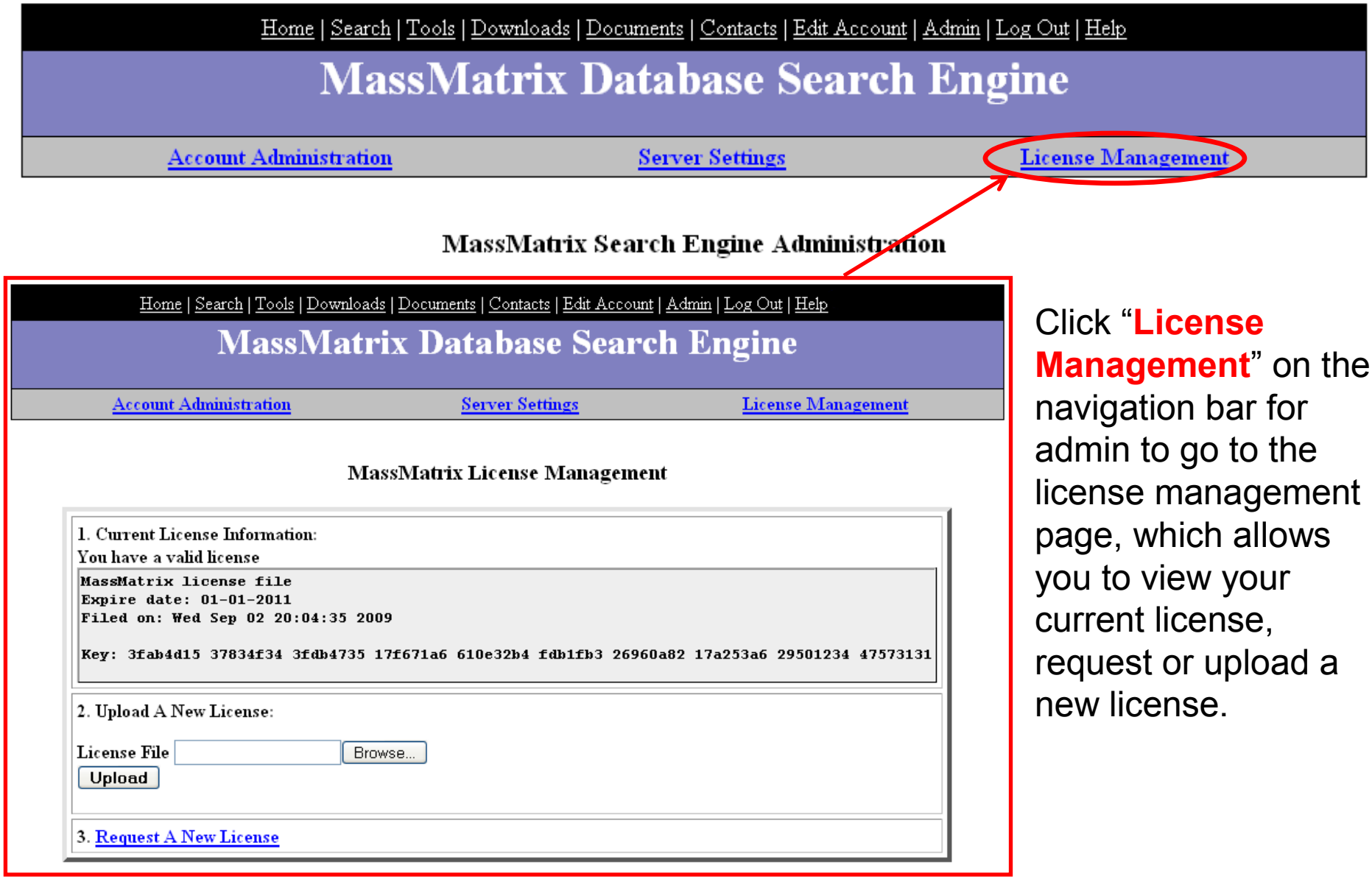


To view search communication log:
Click the "View Search Communication Log" link to view the log of all communications between the head node and the remote compute nodes. *Only available on a cluster*.

comm2nodes.log

```
************************************************************************
* Connection to node1 has been established on Fri Jun 12 13:38:07 2009 *
************************************************************************
************************************************************************
* Connection to node4 has been established on Fri Jun 12 13:38:07 2009 *
************************************************************************
************************************************************************
* Connection to node2 has been established on Fri Jun 12 13:38:08 2009 *
************************************************************************
************************************************************************
* Connection to node1 has been established on Mon Jun 15 10:19:12 2009 *
************************************************************************
************************************************************************
* Connection to node4 has been established on Mon Jun 15 10:19:12 2009 *
************************************************************************
************************************************************************
* Connection to node2 has been established on Mon Jun 15 10:19:13 2009 *
************************************************************************
```

MassMatrix Web Server – License Management



Click "**License Management**" on the navigation bar for admin to go to the license management page, which allows you to view your current license, request or upload a new license.

MassMatrix Web Server – Search Monitor

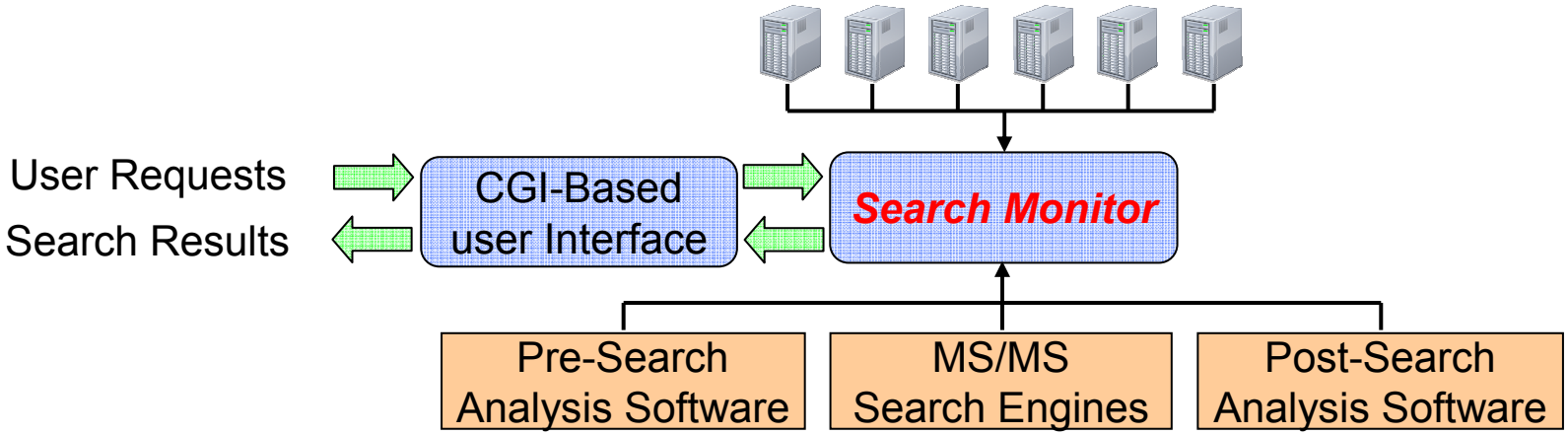
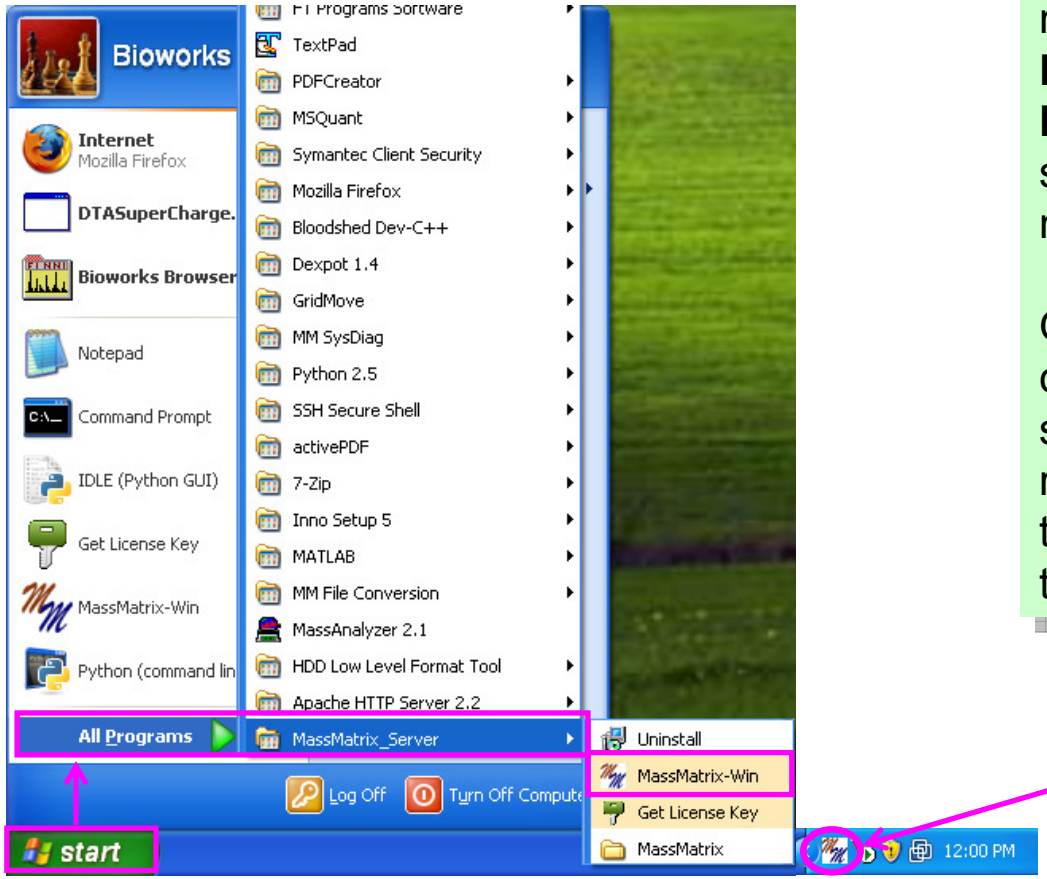


Diagram of MassMatrix Web Server

The search monitor is a perpetually running program, which monitors everything going on the server related to MassMatrix search engine. New search requests from users are submitted to the search monitor by the web interface and processed by the search monitor. All other user and administration activities including deleting results, configurations, and etc are also processed by the search monitor. So it is essential that the search monitor is running all the time. All activities of the search monitor are kept in the search monitor log. Please refer to the section of "**Sever Administration – Search Monitor Log**" for details.

The search monitor is a windows service program or a Linux daemon program. It is designed to start automatically when the system boots and run perpetually without user intervention. Therefore, normally you don't have to worry about it. Under some rare circumstances, such as operating system failure or hardware failure, the search monitor might be terminated. Under those circumstances, you may have to start it manually.

MassMatrix Web Server – Search Monitor (Windows)



On Windows, the search monitor is called "MassMatrix-Win". It is automatically started when windows boots. You can manually start it by clicking on "**start -> All Programs -> MassMatrix Server -> MassMatrix-Win**". After it is started, the search monitor will be automatically minimized to the taskbar.

Only one instance of the search monitor can be running at any time. If you try to start the search monitor when it is actually running, an error message will pop up. But this does not hurt and will not interfere with the running search monitor.

The search monitor is running on the taskbar

MassMatrix Web Server – Search Monitor (Linux)

```
[root@server ~]# massmatrix
Usage: massmatrixd start|stop|restart|status
[root@server ~]# massmatrix status
MassMatrix daemon is running.
[root@server ~]# massmatrix stop
[root@server ~]# massmatrix status
MassMatrix daemon is not running.
[root@server ~]# massmatrix restart
MassMatrix daemon not running.
[root@server ~]# massmatrix start
MassMatrix daemon already running.
[root@server ~]# massmatrix status
MassMatrix daemon is running.
[root@server ~]#
```

On Linux, the search monitor is called “massmatrix” unless you changed it during installation. The search monitor is a Linux daemon program. It will automatically run in background when Linux boots. You may also manually check its status, stop, start, and restart it. **The search monitor should be run by root account or “sudo” command.**

Usage of the search monitor:
1. massmatrix status
Check the status of search monitor

2. massmatrix start
Start search monitor

3. massmatrix stop
Stop search monitor

4. massmatrix restart
Restart search monitor

MassMatrix Web Server – HTTP Server

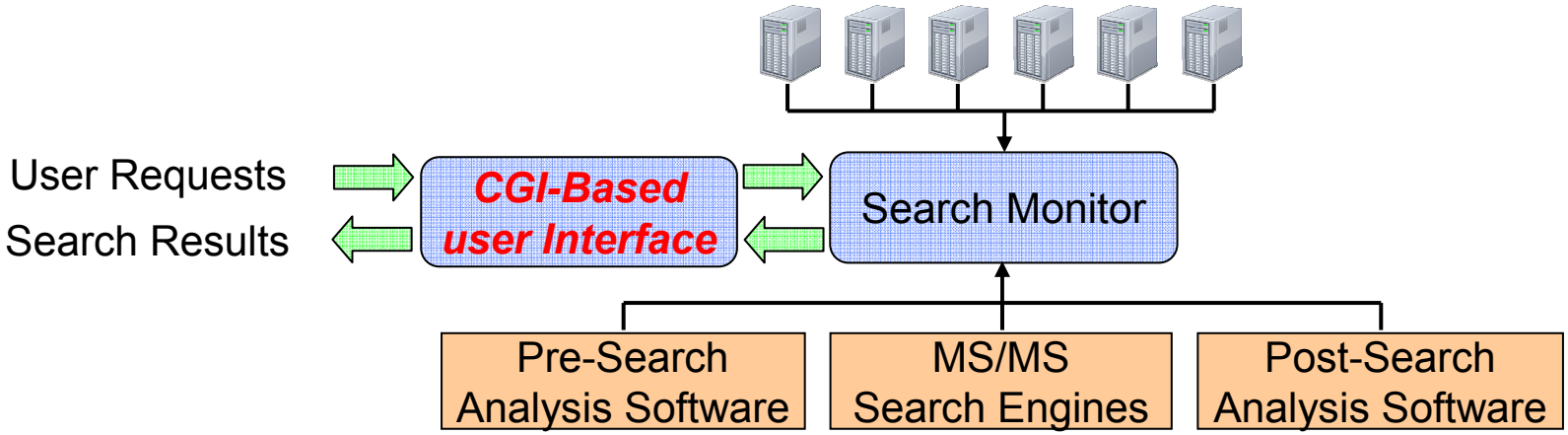
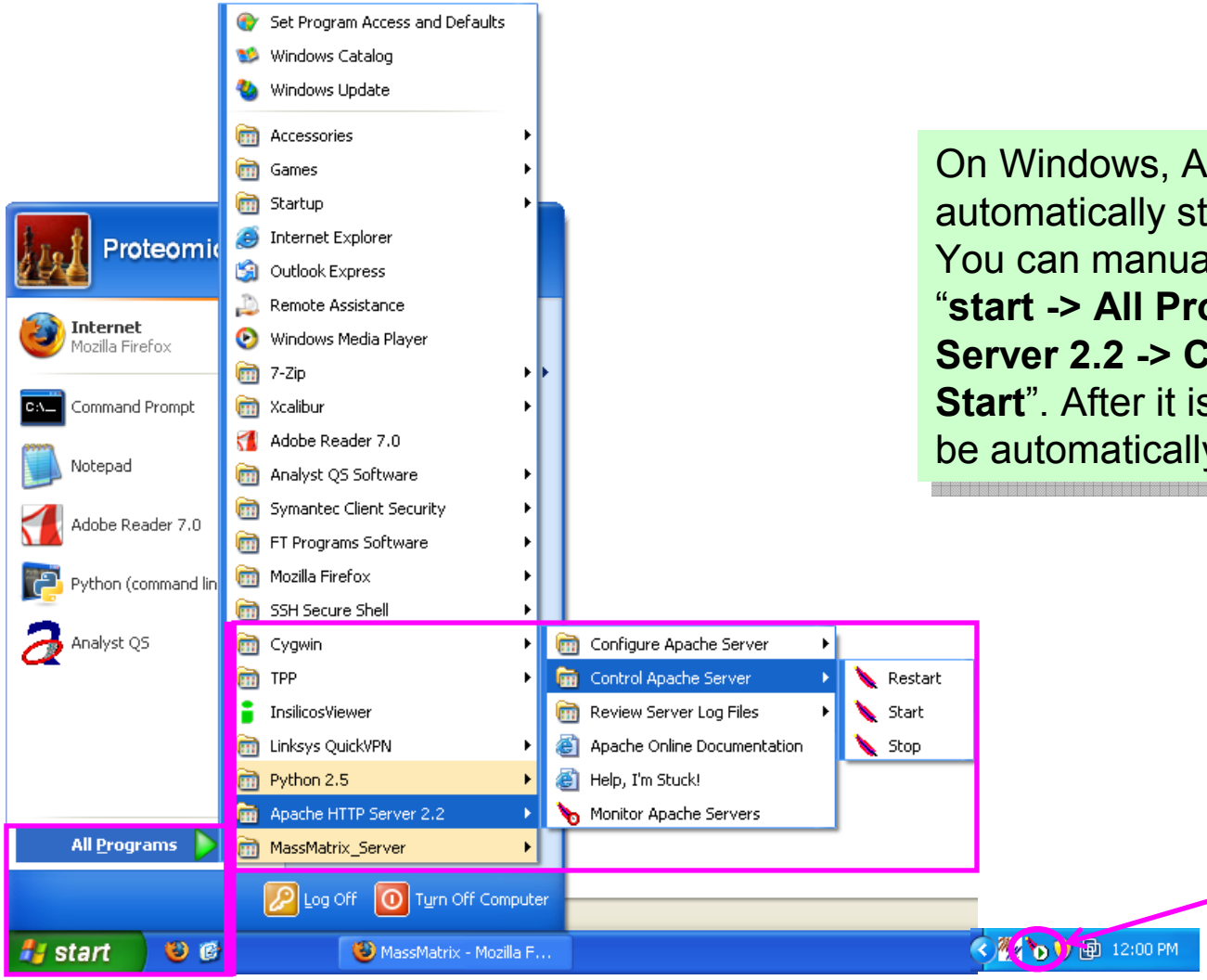


Diagram of MassMatrix Web Server

While the mass search monitor serves the search engine and all other related data analysis software, the HTTP server serves the web interface to users. So it is also essential that the HTTP server is running all the time.

Apache 2.2 is used as HTTP server on windows. It is a windows service program. On Linux, the HTTP server comes with the Linux distribution. They are designed to start automatically when the system boots and run perpetually without user intervention. Therefore, normally you don't have to worry about them. Under some rare circumstances, such as operating system failure or hardware failure, a HTTP server might be terminated. Under those circumstances, you may have to start it manually.

MassMatrix Web Server – Apache 2.2 (Windows)



On Windows, Apache 2.2 HTTP server is automatically started when windows boots. You can manually start it by clicking on "**start -> All Programs -> Apache HTTP Server 2.2 -> Control Apache Server -> Start**". After it is started, Apache 2.2 will be automatically minimized to the taskbar.

Apache 2.2 is running on the taskbar

MassMatrix Web Server – HTTP Server (Linux)

```
[root@massmatrix ~]# service httpd
Usage: httpd {start|stop|restart|condrestart|reload|status|fullstatus|graceful|h
elp|configtest}
[root@massmatrix ~]# service httpd status
httpd (pid 8751 8750 8749 8748 8747 8746 8745 8744 8742) is running...
[root@massmatrix ~]# service httpd stop
Stopping httpd:                                            [  OK  ]
[root@massmatrix ~]# service httpd start
Starting httpd: httpd: apr_sockaddr_info_get() failed for massmatrix
httpd: Could not reliably determine the server's fully qualified domain name, us
ing 127.0.0.1 for ServerName
                                                           [  OK  ]
[root@massmatrix ~]# service httpd restart
Stopping httpd:                                            [  OK  ]
Starting httpd: httpd: apr_sockaddr_info_get() failed for massmatrix
httpd: Could not reliably determine the server's fully qualified domain name, us
ing 127.0.0.1 for ServerName
                                                           [  OK  ]
```

On Linux, the http sever comes with the Linux distribution. It is a Linux daemon program. It will automatically run in background when Linux boots. You may also manually check its status, stop, start, and restart it by the following commands:

service httpd [status | stop | start | restart]
or
service apache2 [status | stop | start | restart]

3. General User Manual

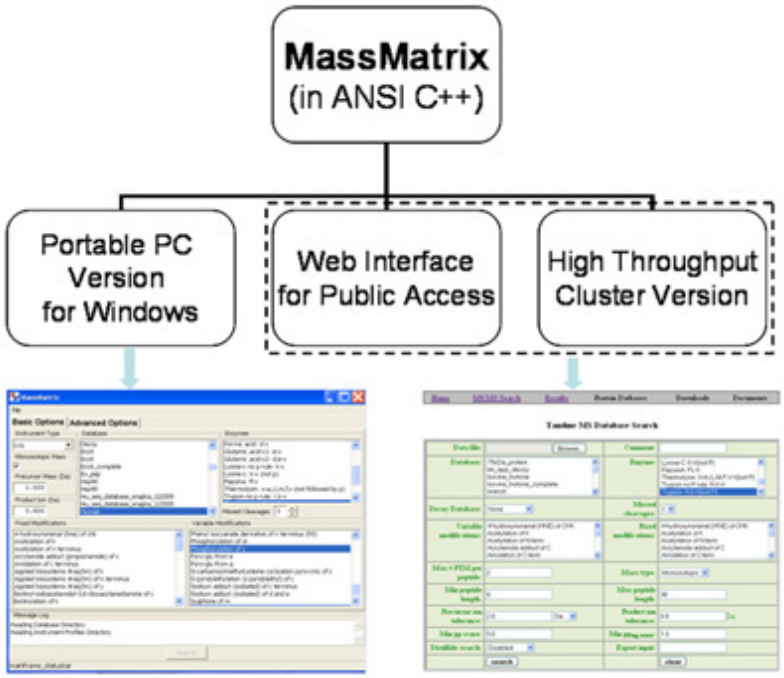
MassMatrix Web Server

Main Navigation Bar of the Site

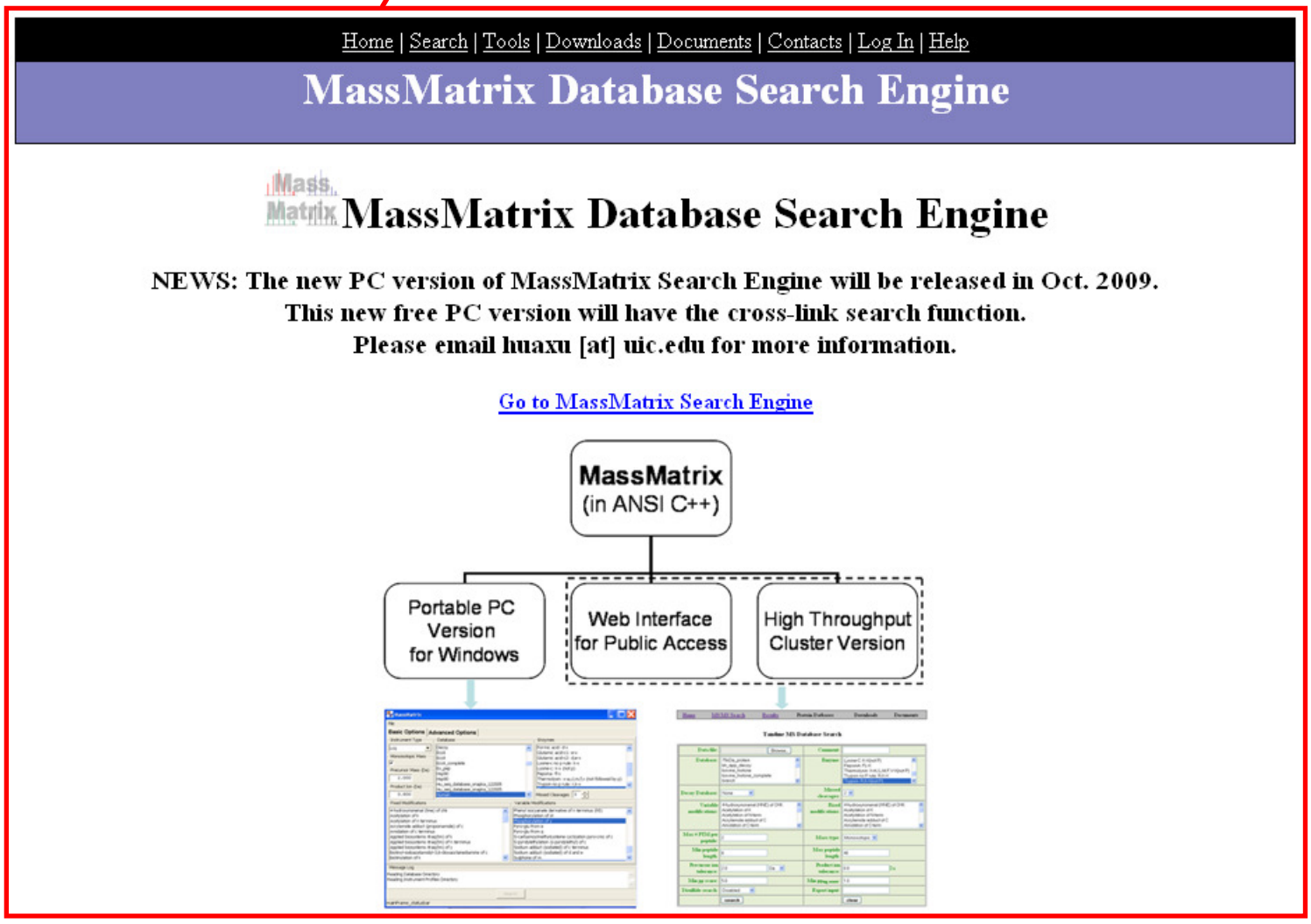
MassMatrix Database Search Engine

assMatrix Search Engine will be released in Oct. 2009.
ion will have the cross-link search function.
Please email huaxu [at] uic.edu for more information.

Go to MassMatrix Search Engine

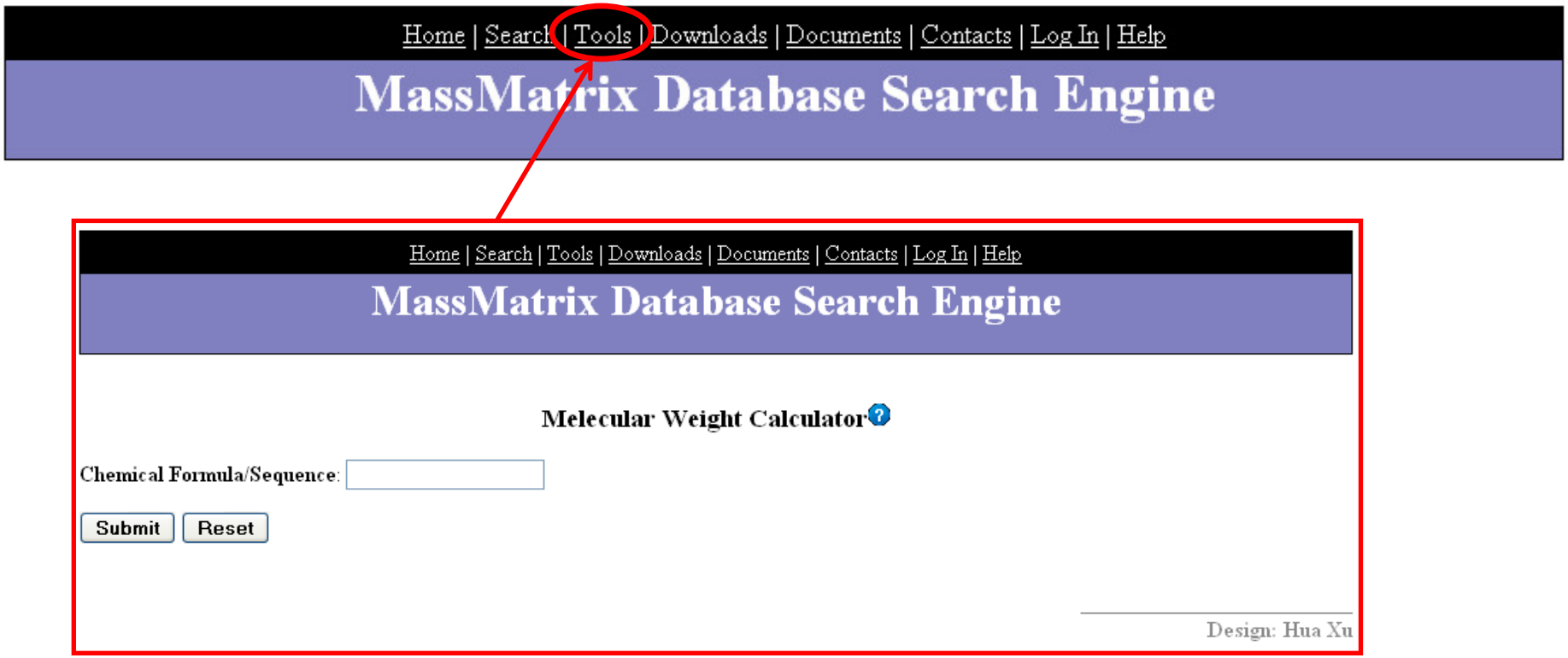


MassMatrix Web Server – Home Page



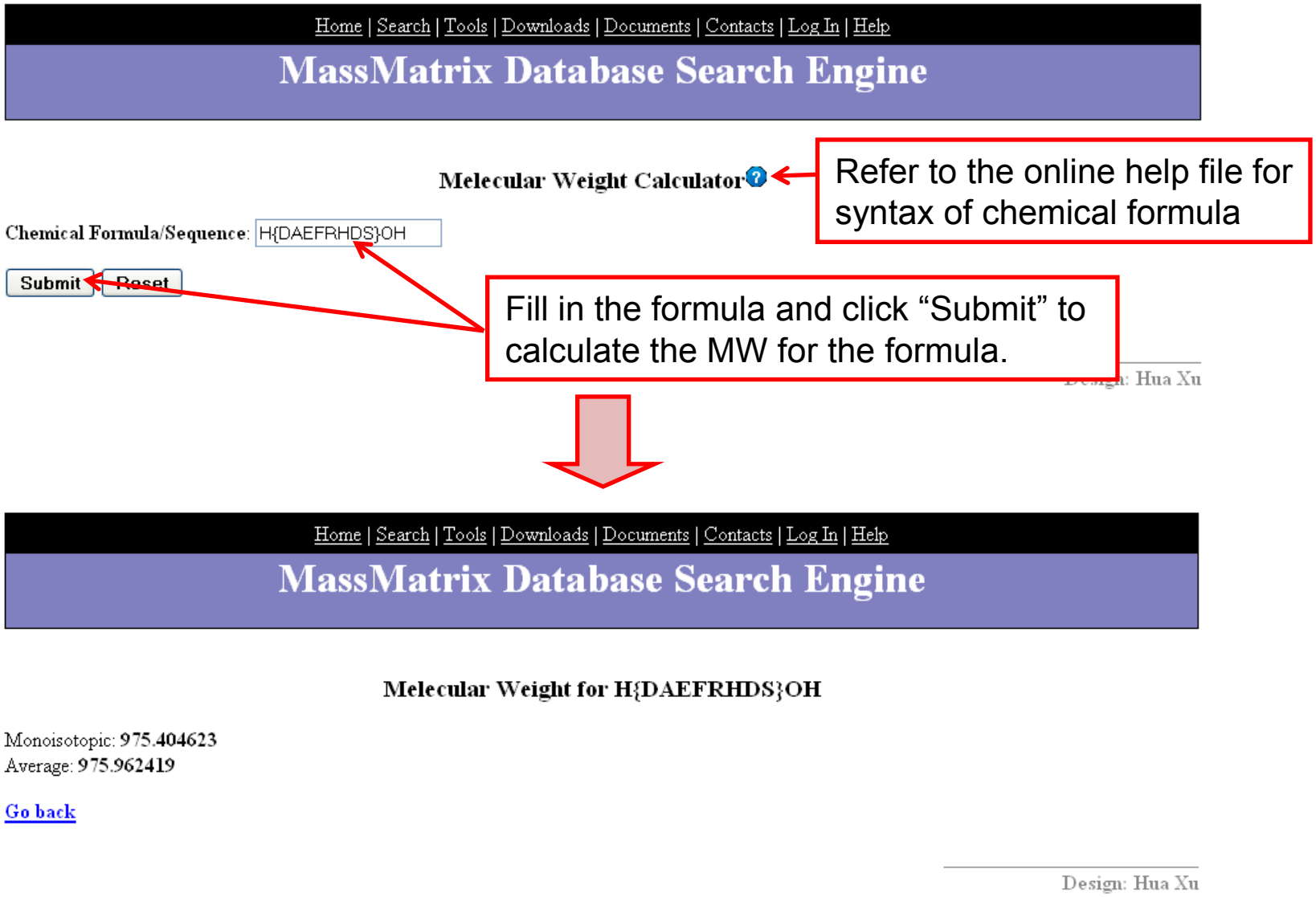
Click "**Home**" on the main navigation bar to go to the home page. It has the general description of MassMatrix search engine.

MassMatrix Web Server – Tools Page



Click "**Tools**" on the main navigation bar to go to the tools page. It currently only has the molecular weight calculator.

MassMatrix Web Server – Tools Page (Con't)



MassMatrix Web Server – Downloads Page

Home | Search | Tools | Downloads | Documents | Contacts | Log In | Help

MassMatrix Database Search Engine

MassMatrix Downloads

1. **MassMatrix PC Beta 1**:
This public beta version of MassMatrix for PC is free for non-commercial use.
To obtain a current licensed copy follow the directions below.
1. Download MassMatrix PC using the above link.
2. Install to the folder C:\Program Files\MassMatrix\
3. Under the program group run "Get License Key" to obtain a unique machine ID.
4. Email the machine ID to license(at)massmatrix.net. In your license request, please include your NAME, TITLE, INSTITUTION/COMPANY, and EMAIL ADDRESS. If you want to install several copies of MassMatrix on several computers, please email the machine IDs for all computers in separate emails.
5. Upon receiving you license file, intall it on your computer.
6. Only three protein databases are included with the installer. You can add databases (FASTA format) to the folder C:\Program Files\MassMatrix\databases.

2. **MM Result Extract**:
The tool is used to extract MassMatrix search results to a spreadsheet.

3. **MM File Conversion Tools**:
These tools convert between common input formats: .RAW (Thermo), .mzXML, .MGF.
Important nodes for Mascot users:
Due to the fact that Mascot doesn't search spectra with charges >= +8, it will sometimes warn you that invalid charges are found in your MGF files during searching. Please ignore the warning and it will not negativelly affect your results. Those spectra with charges >= +8, however, are real spectra with high charges and can be positively identified in other search programs, such as MassMatrix.

4. **MM Automated LC-MS/MS System Diagnostic Tool**:
MassMatrix LC-MS/MS system diagnosis provides fully automated and real-time system diagnosis. The program is built based on MassMatrix database search program (www.massmatrix.net). The current release is targeted to system diagnosis of LCQ, LTQ, LTQ-Orbitrap and LTQ-FTICR mass spectrometers (ThermoFinigan, CA, USA).

5. **Tools for Generating Decoy Protein Databases**:
These tools are used to generate decoy protein databases (reversed or randomized protein sequences from the original protein databases).

6. **MassMatrix Retention Time Analysis**:
This public beta version of MassMatrix LR_RT is free for non-commercial use. Use this program to score peptide matches from database search programs (MassMatrix, Mascot, X!Tandem and among others) based on their predicted retention time.

7. **MassMatrix Noise Filtering**:
This public beta version of MassMatrix DNL is free for non-commercial use. Use this program to filter noise from LC-MS/MS spectra.

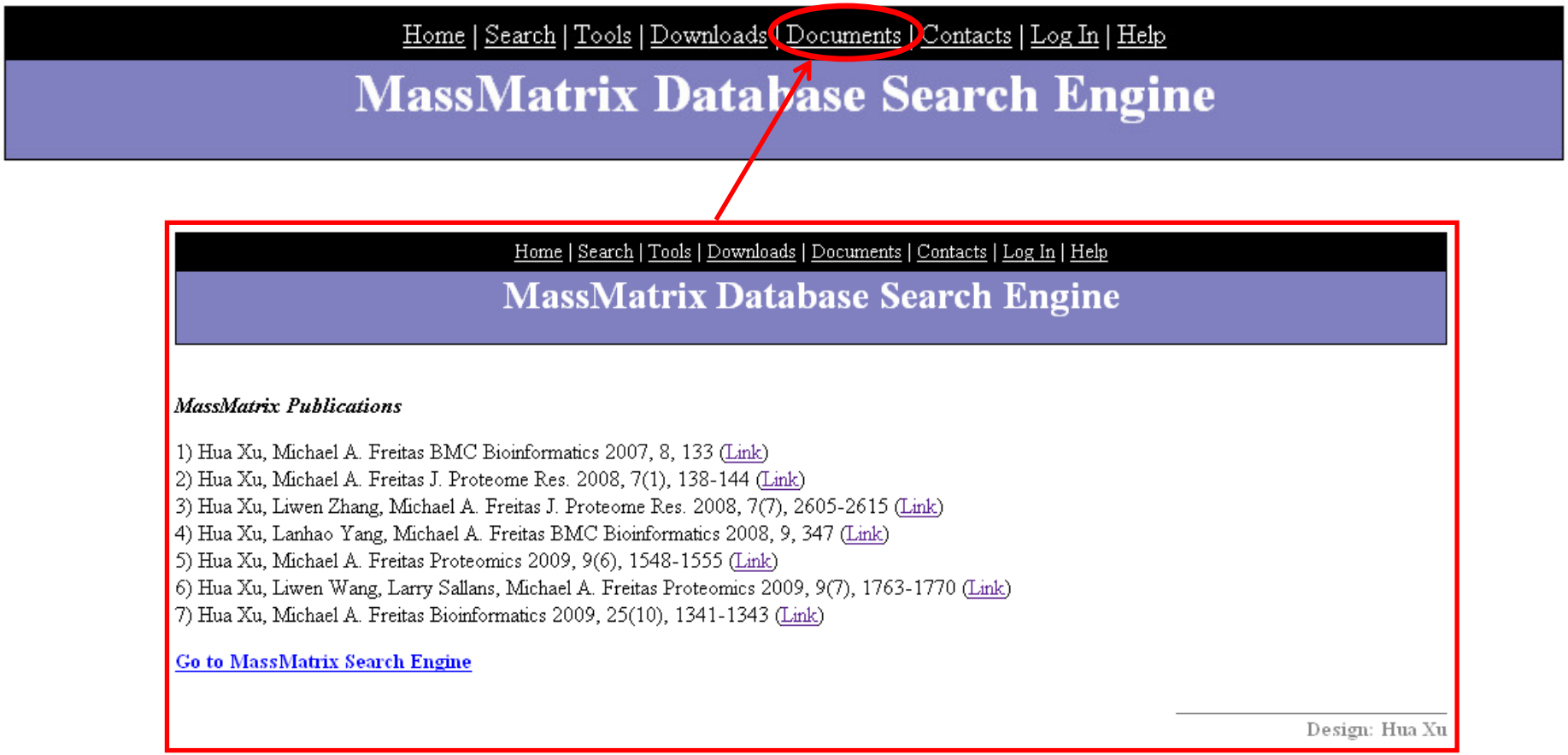
8. **Quantitation Analysis for N15 Labeling**:
The software is under beta testing, for more information, please contact Hua Xu at huaxu@uic.edu.

9. **Multiple-Way Comparison**:
The software is under beta testing, for more information, please contact Hua Xu at huaxu@uic.edu.

Click "**Downloads**" on the main navigation bar to go to the downloads page. It has may data analysis programs and tools for mass spectrometric data of proteins and peptides.

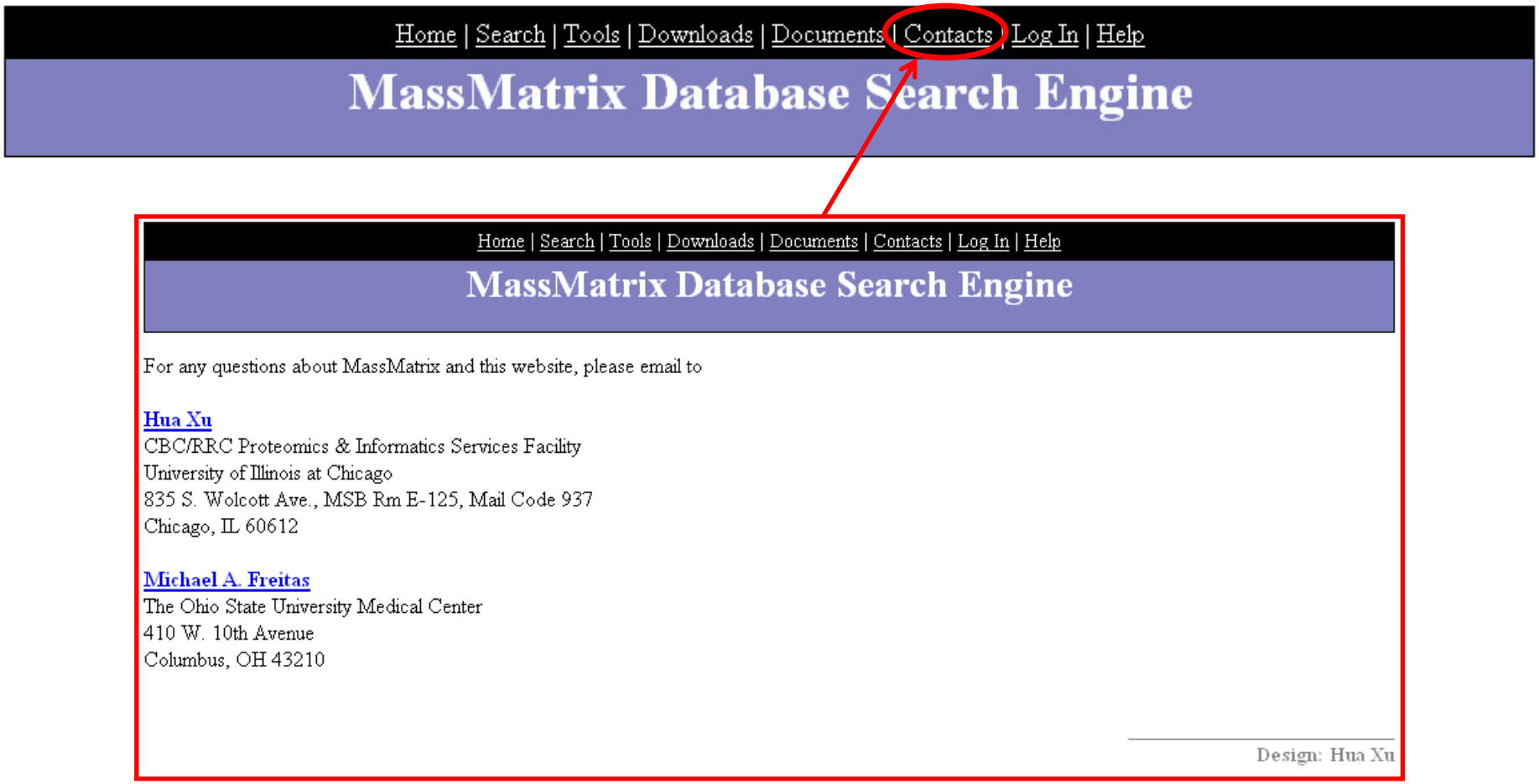
Pleas read the instructions carefully and click on the links to download the programs and tools.

MassMatrix Web Server – Documents Page



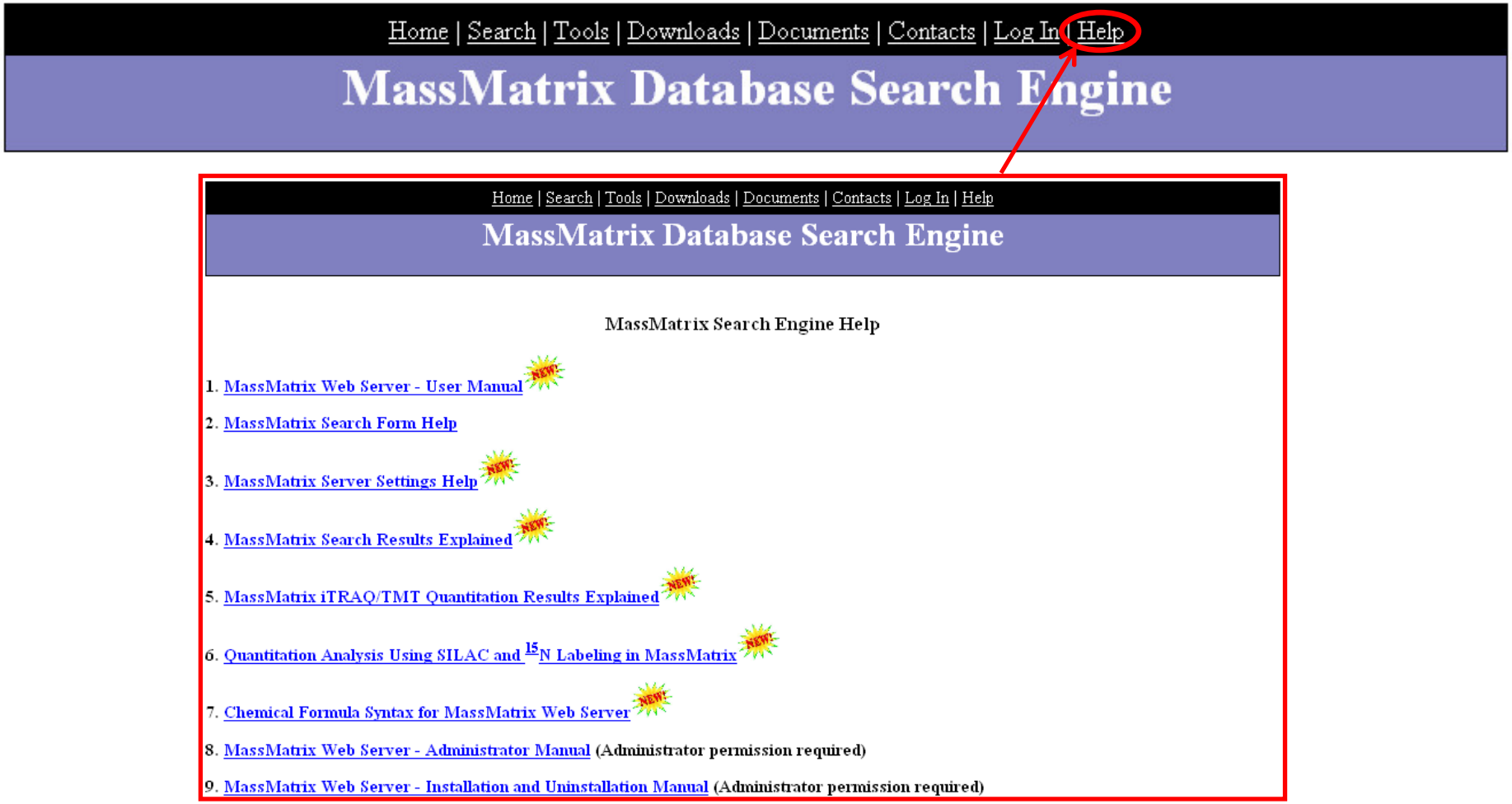
Click "**Documents**" on the main navigation bar to go to the documents page. It has the list of publications about MassMatrix database search engine. Click on the links to go to the publications at http://www.ncbi.nlm.nih.gov/pubmed/.

MassMatrix Web Server – Contacts Page



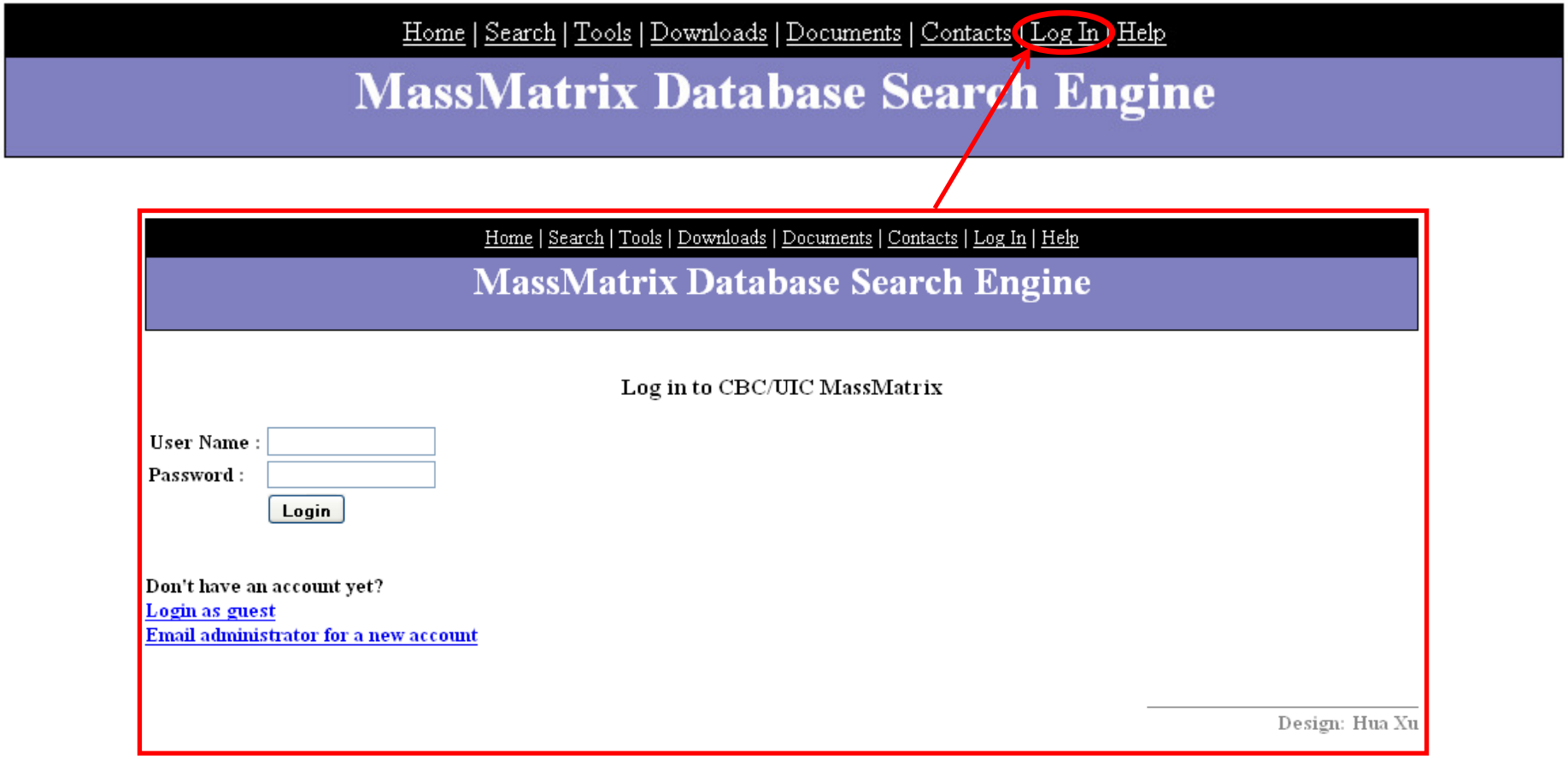
Click "**Contacts**" on the main navigation bar to go to the contacts page. It has the contact information that you need for any questions and feature requests about MassMatrix.

MassMatrix Web Server – Help Page



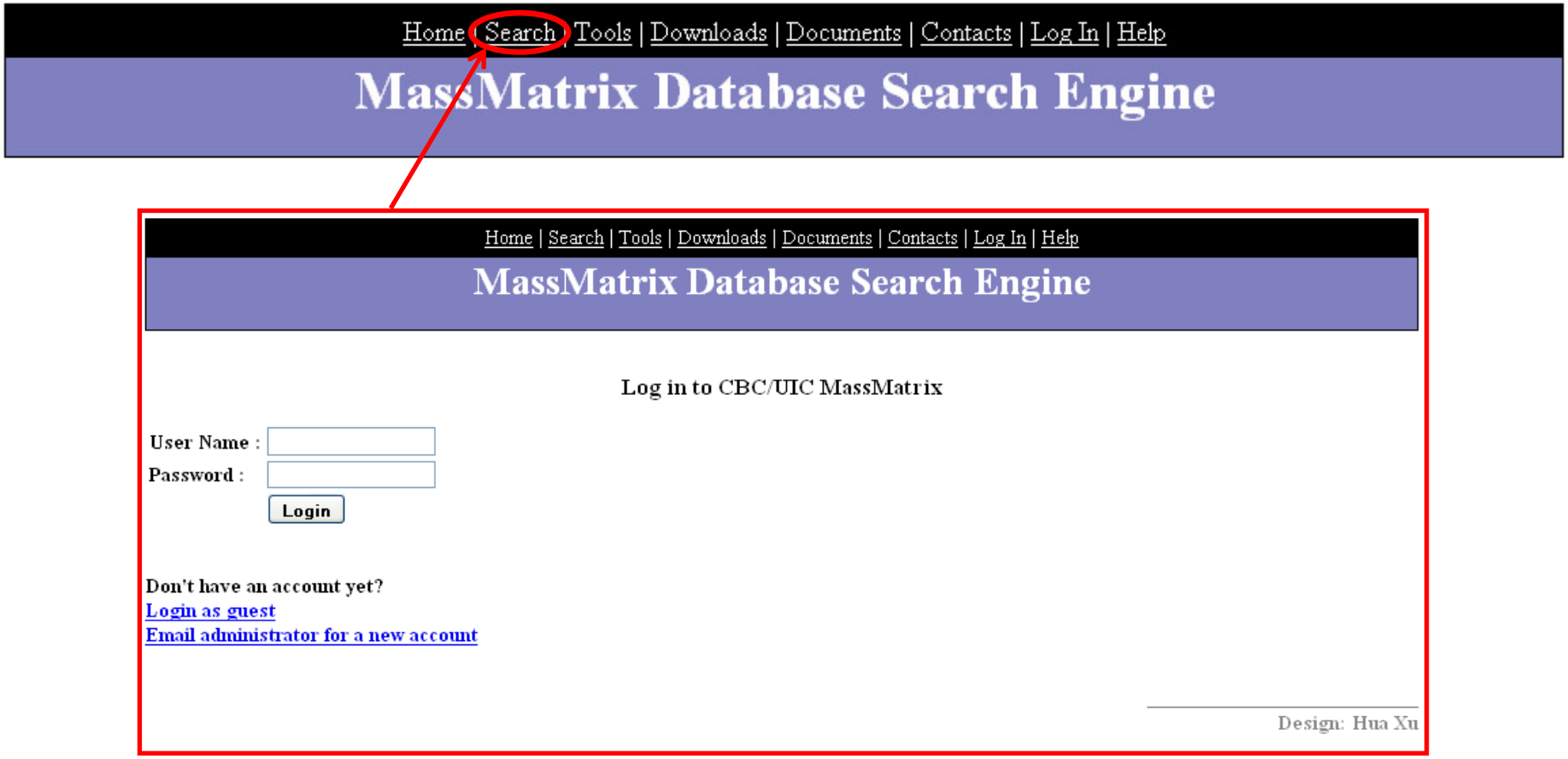
Click "**Help**" on the main navigation bar to go to the help page. The help page has all the online help files. Click on the links to go to those online help files. Online help files are import resources about how to use and configure the online server and how to interpret your results.

MassMatrix Web Server – Login Page



Click "**Log In**" on the main navigation bar to go to the login page. You will have to log in in order to use the database search engine to perform data analysis.

MassMatrix Web Server – Search Engine

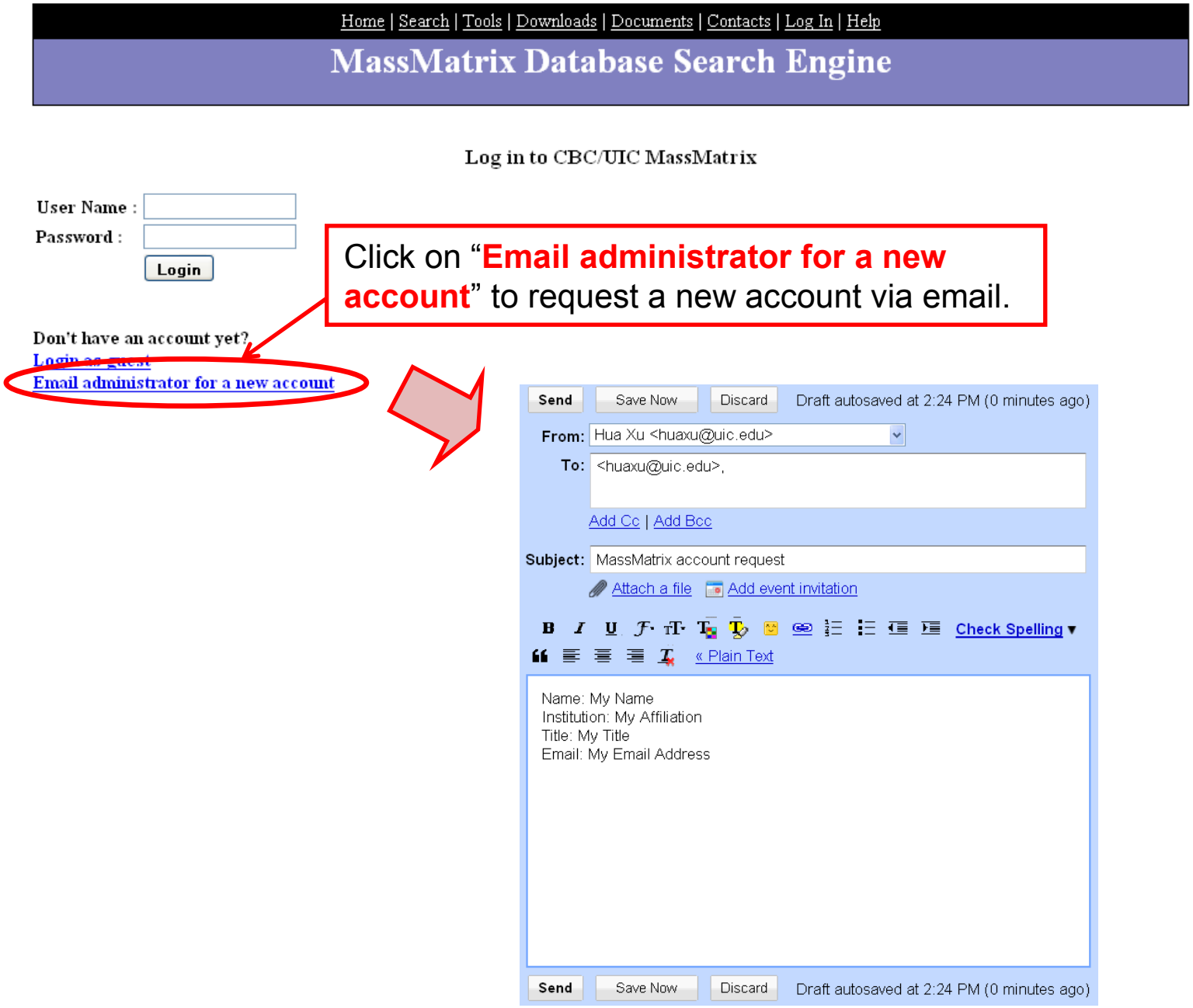


You have to log in in order to use the search engine. Clicking "**Search**" on the main navigation bar will direct you to the login page **if you are not logged in**.

MassMatrix Web Server – Guest Login

Guest login is only used for you to do a quick test of MassMatrix. It is not recommended to log in as guest if you want to do real searches. The search results, uploaded protein databases, and data sets under the guest account are accessible to anyone who logs in as guest. Fore security purpose, please request a new account to make your search results, protein databases and data sets only accessible to you through your account (see next slide).

MassMatrix Web Server – Request An Account



MassMatrix Web Server – Login

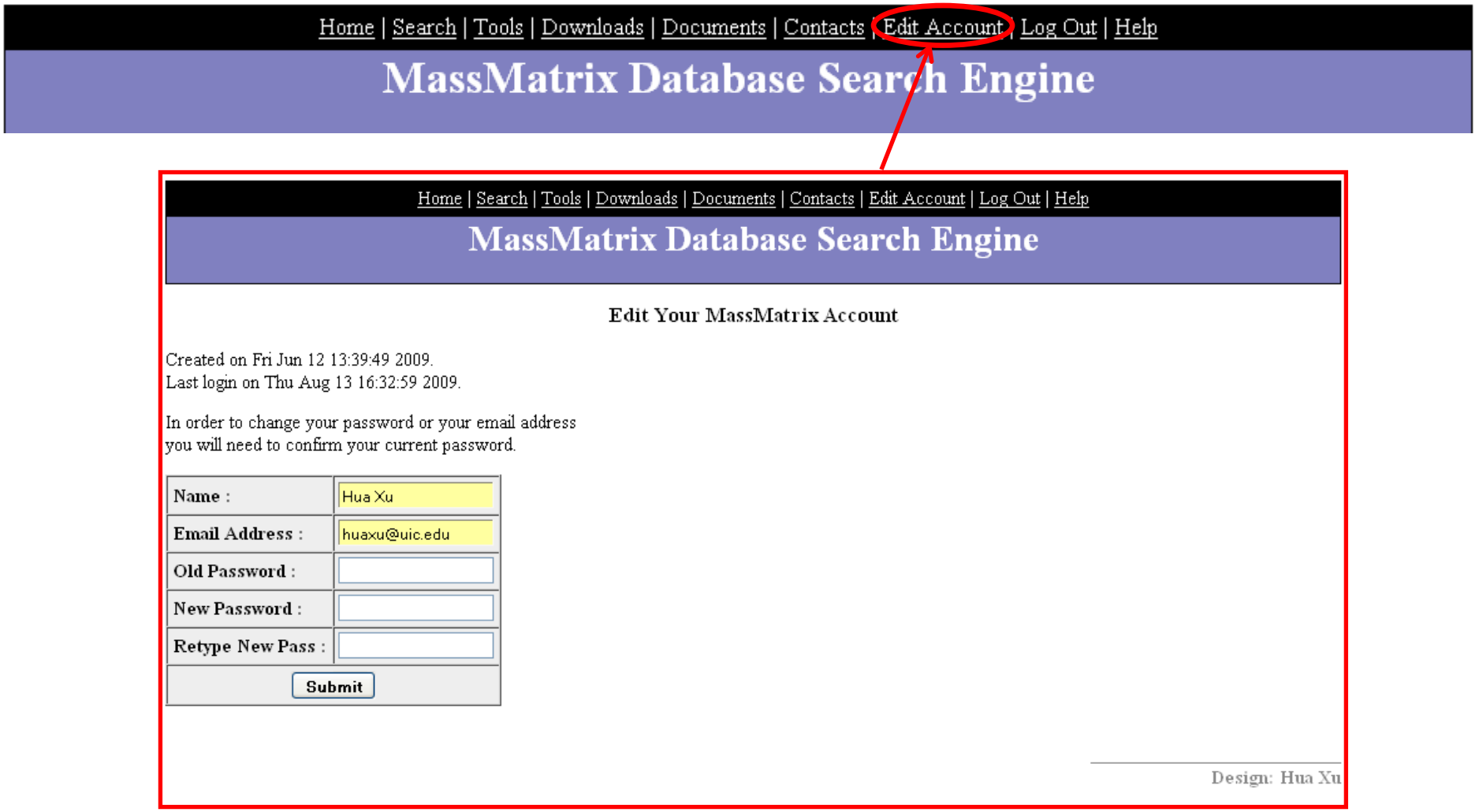
MassMatrix Web Server – After Login

After you are logged in, the main navigation bar changes accordingly.

The "Log In" changes to "Log Out", which allows you to log out the server.

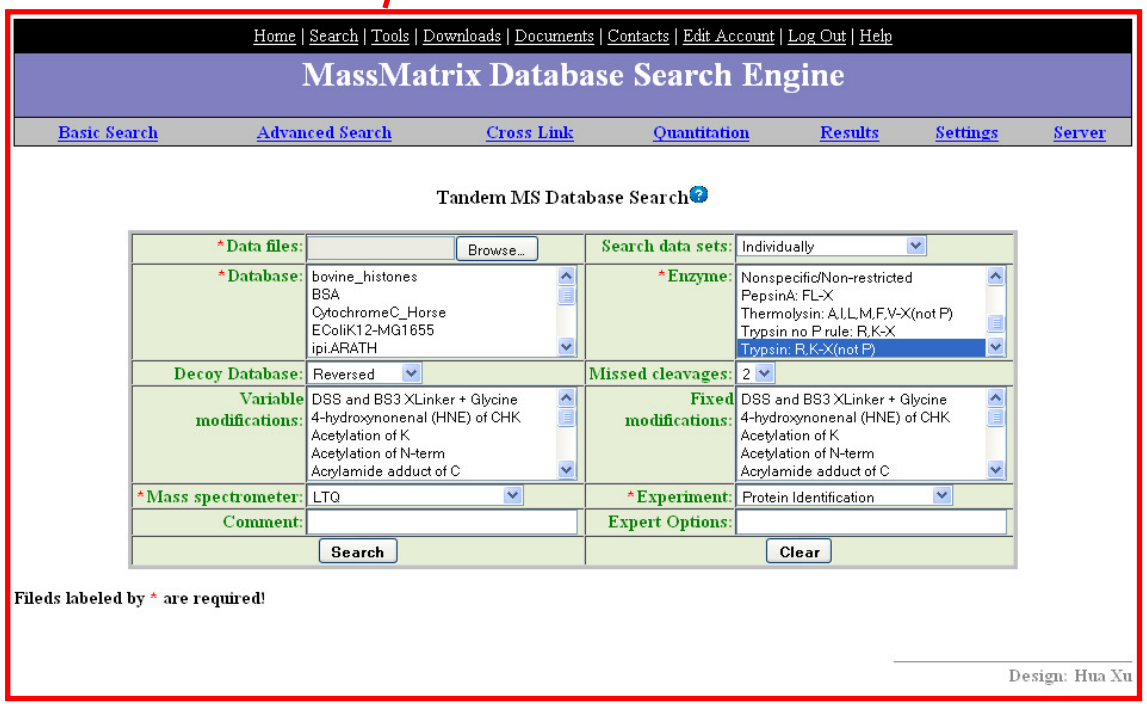
A new link to editing your account appears unless you are logged in as Guest.

MassMatrix Web Server – Edit Account



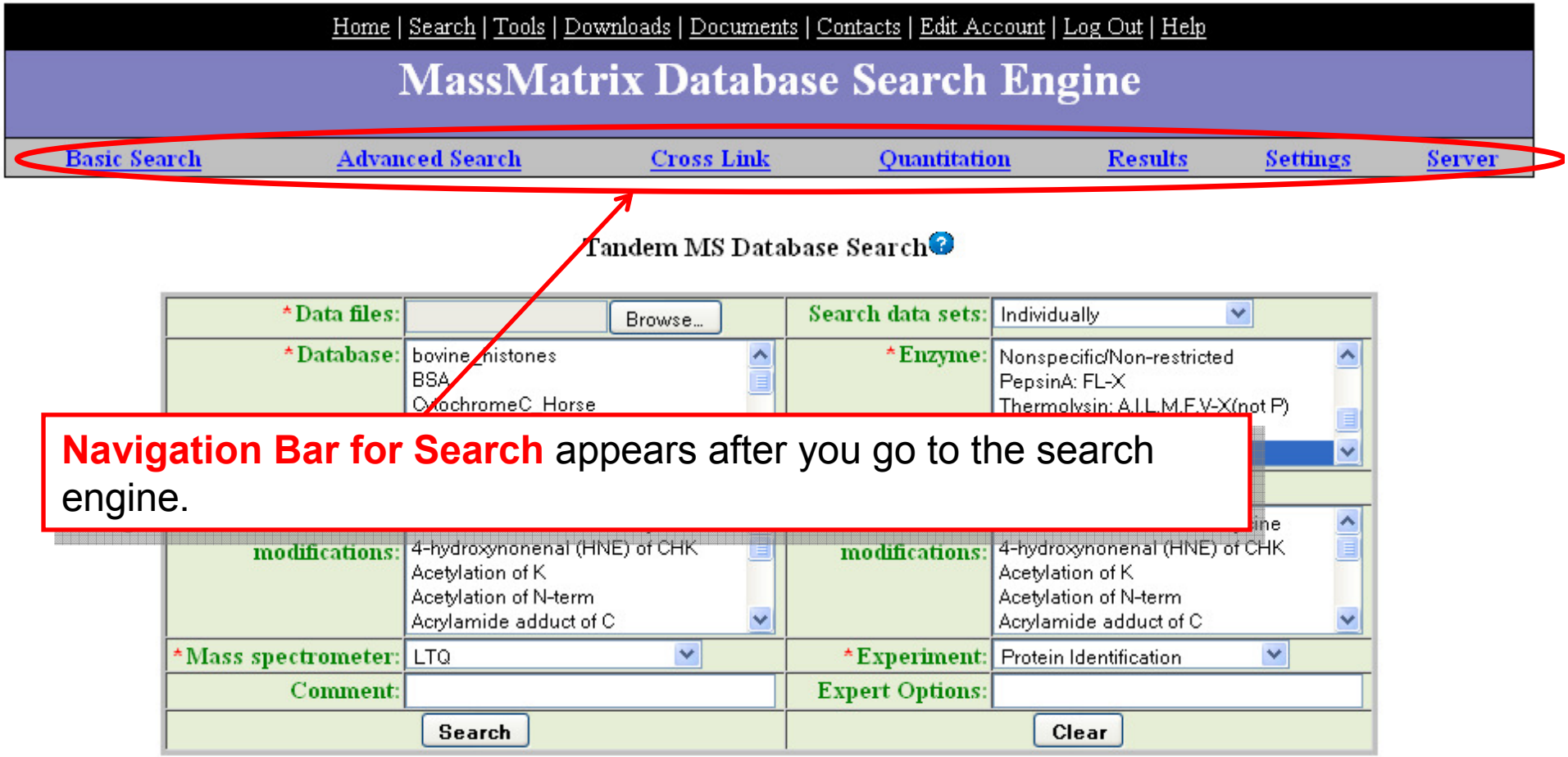
Click "**Edit Account**" on the main navigation bar to go to the account editing page (**Not available for Guest**). You may change your name, email address, and password. Old password has to be provided in order to make changes.

MassMatrix Web Server – Go to Search Engine



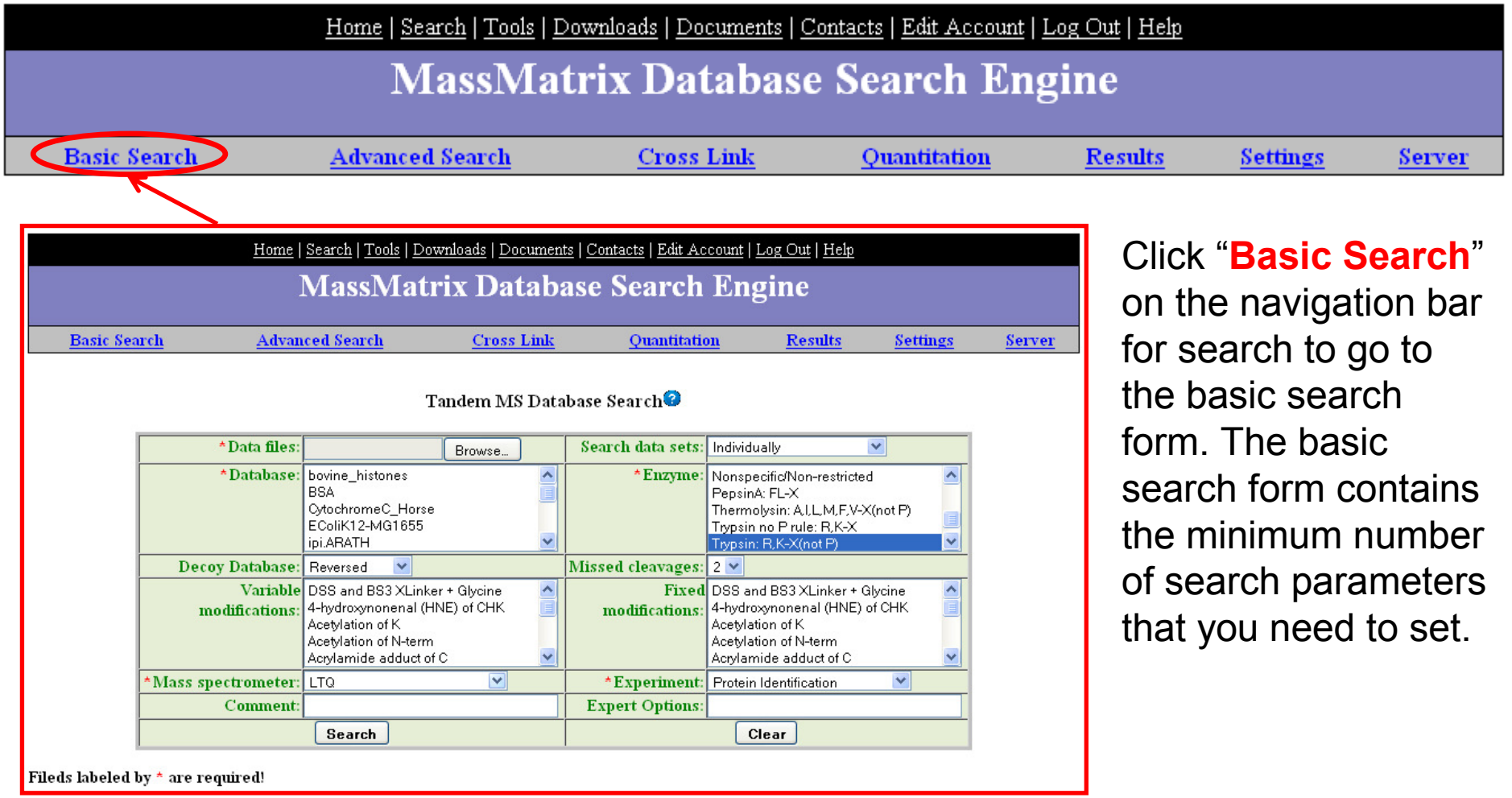
After you are logged in, click "**Search**" on the main navigation bar to go to the search engine.

MassMatrix Search Engine



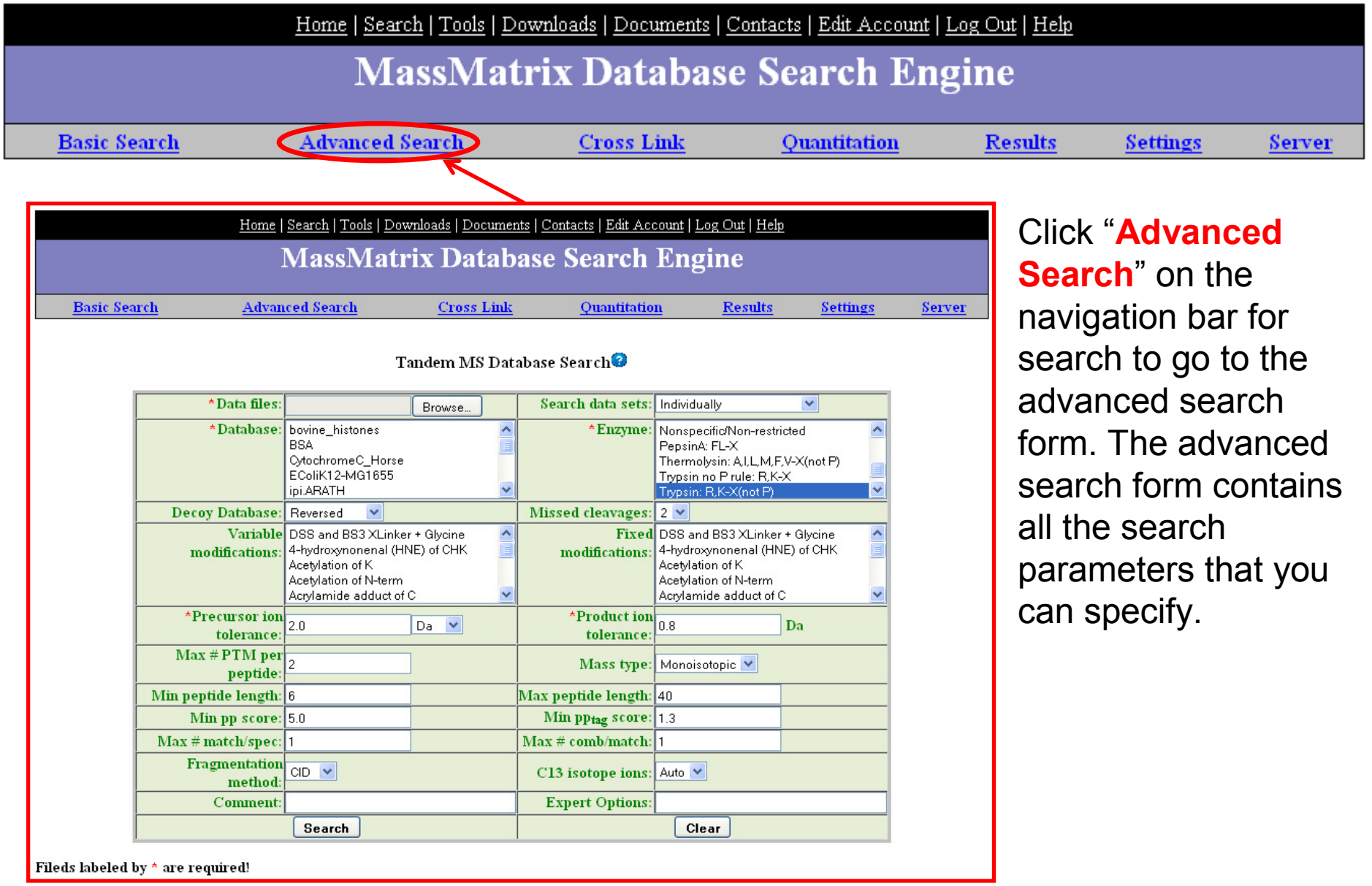
Navigation Bar for Search appears after you go to the search engine.

MassMatrix Search Engine – Basic Search



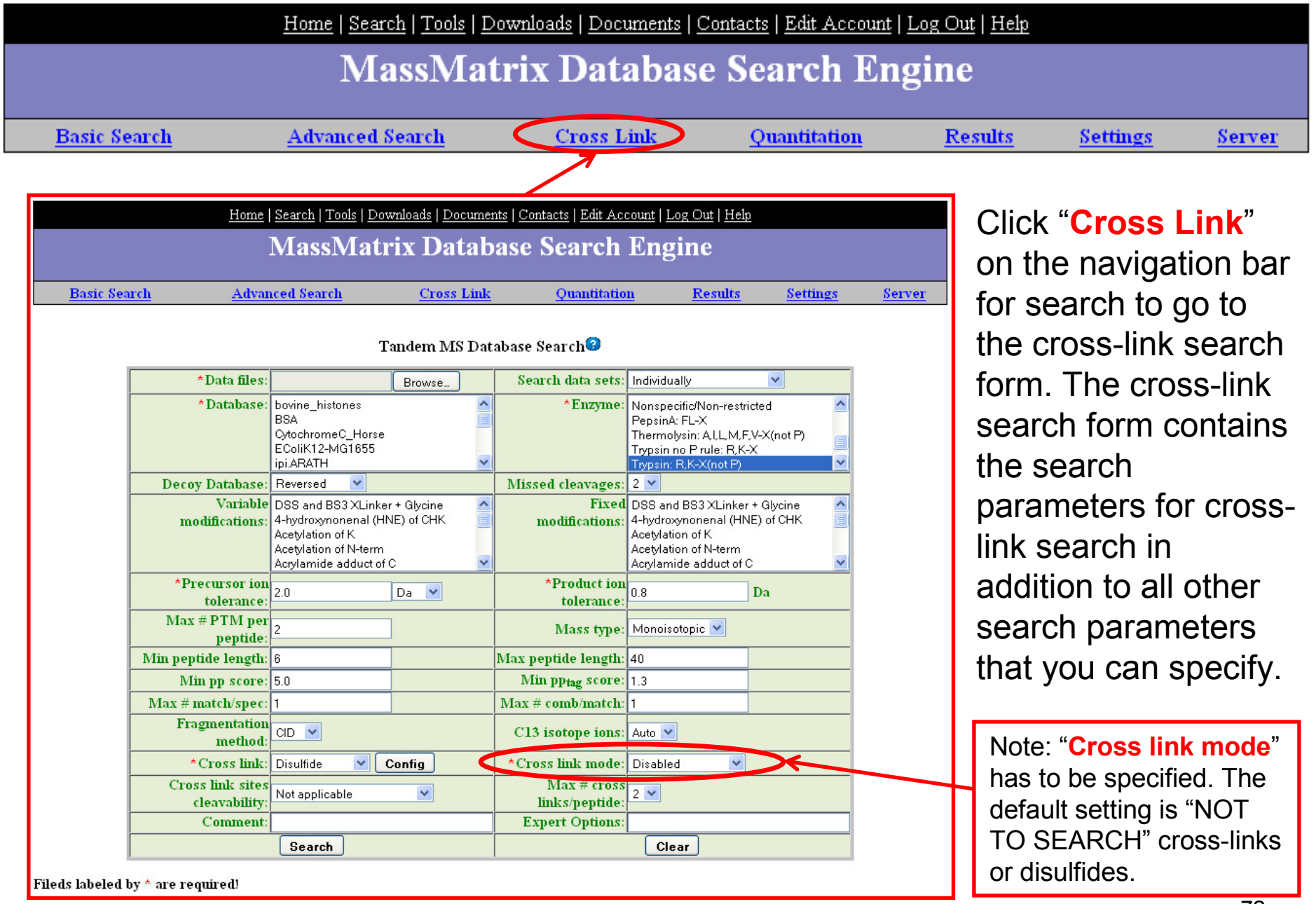
Click "**Basic Search**" on the navigation bar for search to go to the basic search form. The basic search form contains the minimum number of search parameters that you need to set.

MassMatrix Search Engine – Advanced Search



Click "**Advanced Search**" on the navigation bar for search to go to the advanced search form. The advanced search form contains all the search parameters that you can specify.

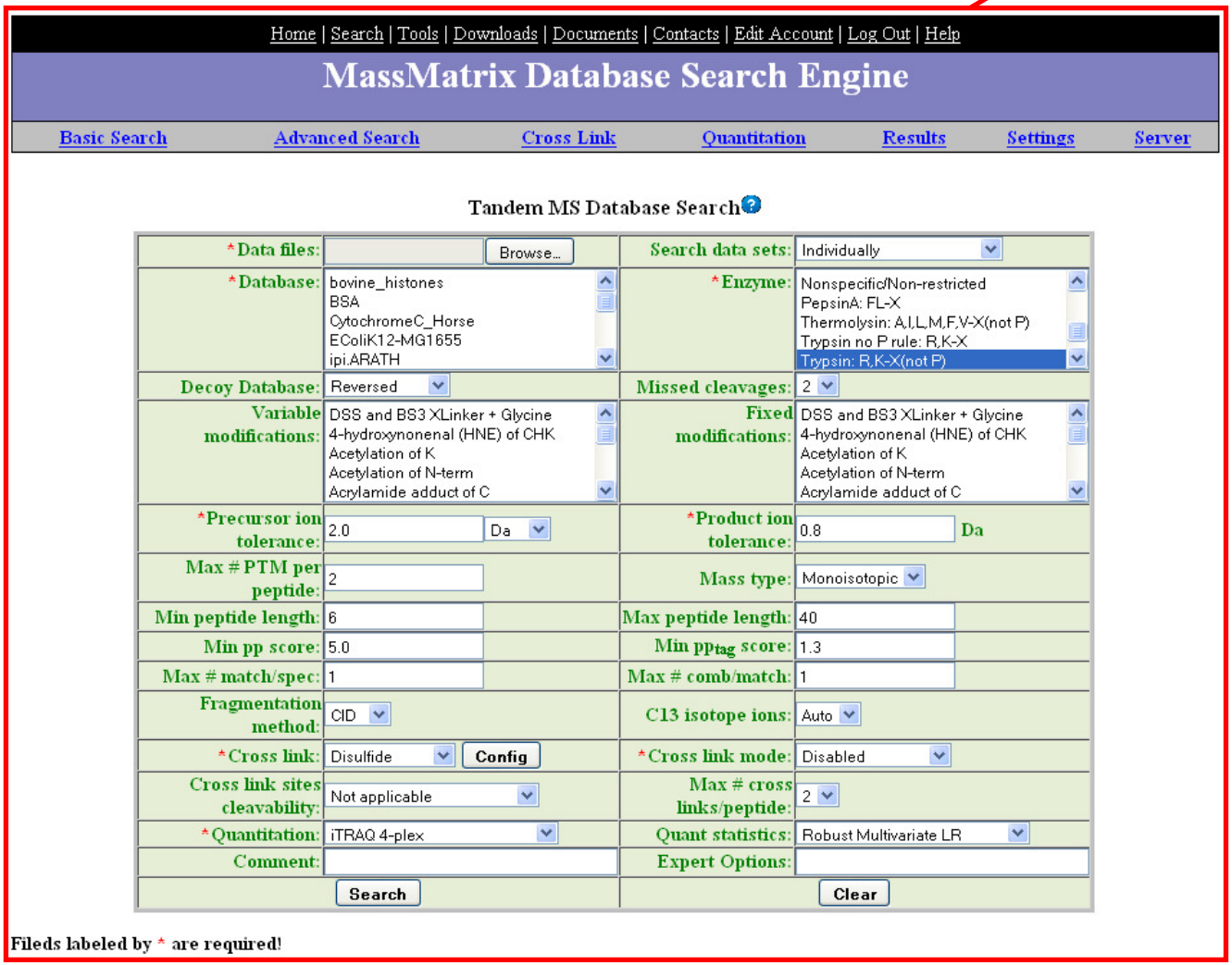
MassMatrix Search Engine – Cross Link Search



Click "**Cross Link**" on the navigation bar for search to go to the cross-link search form. The cross-link search form contains the search parameters for cross-link search in addition to all other search parameters that you can specify.

Note: "**Cross link mode**" has to be specified. The default setting is "NOT TO SEARCH" cross-links or disulfides.

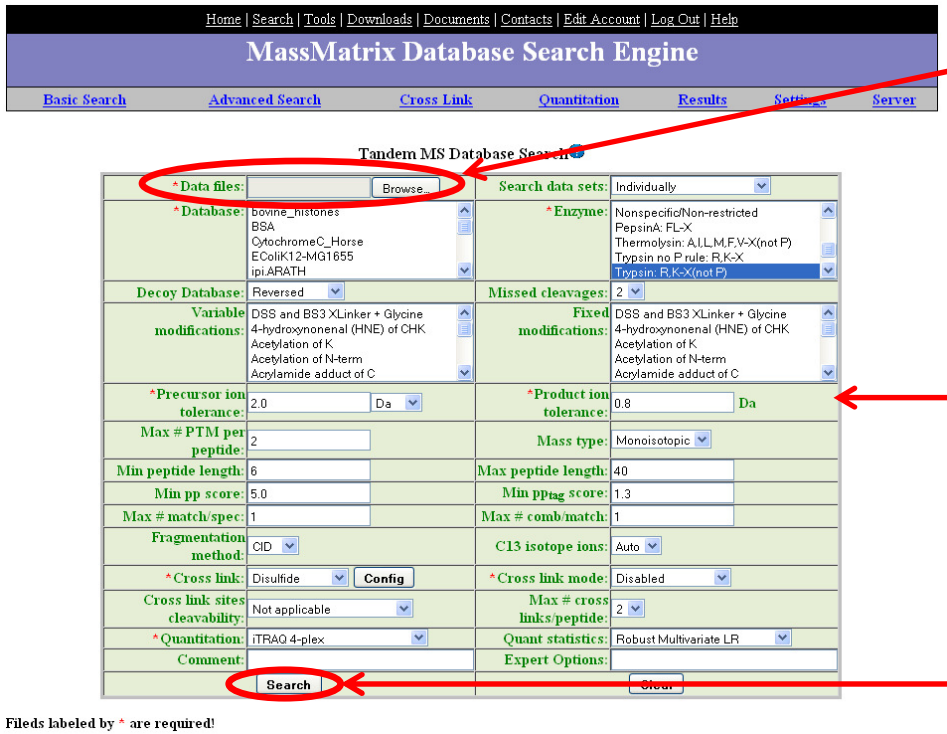
MassMatrix Search Engine – Quantitation



Click "**Quantitation**" on the navigation bar for search to go to the quantitation search form. The quantitation search form contains the quantitation parameters in addition to all the search parameters that you can specify. Quantitation methods currently supported:

1) **iTRAQ and TMT**;
2) **^{15}N labeling**;
3) **SILAC**.

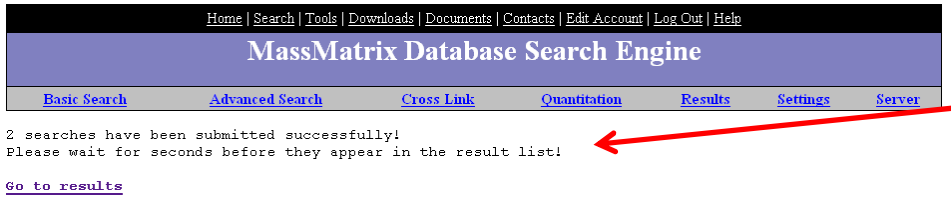
MassMatrix Search Engine – Search Submission



Step 1: Click "**Browse**" icon to choose a data file that you want to search.
Note: Multiple data files can be selected by repeating step 1.

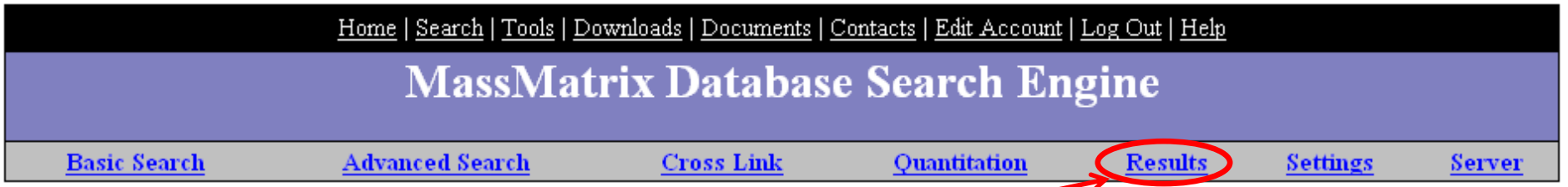
Step 2: Specify all the search parameters and quantitation parameters (if you are doing quantitation searches) in the search form. Please refer to the online help file "MassMatrix Search Form Help" for the details about all the parameters. See "Help Page" Section of this manual for how to access the online help file.

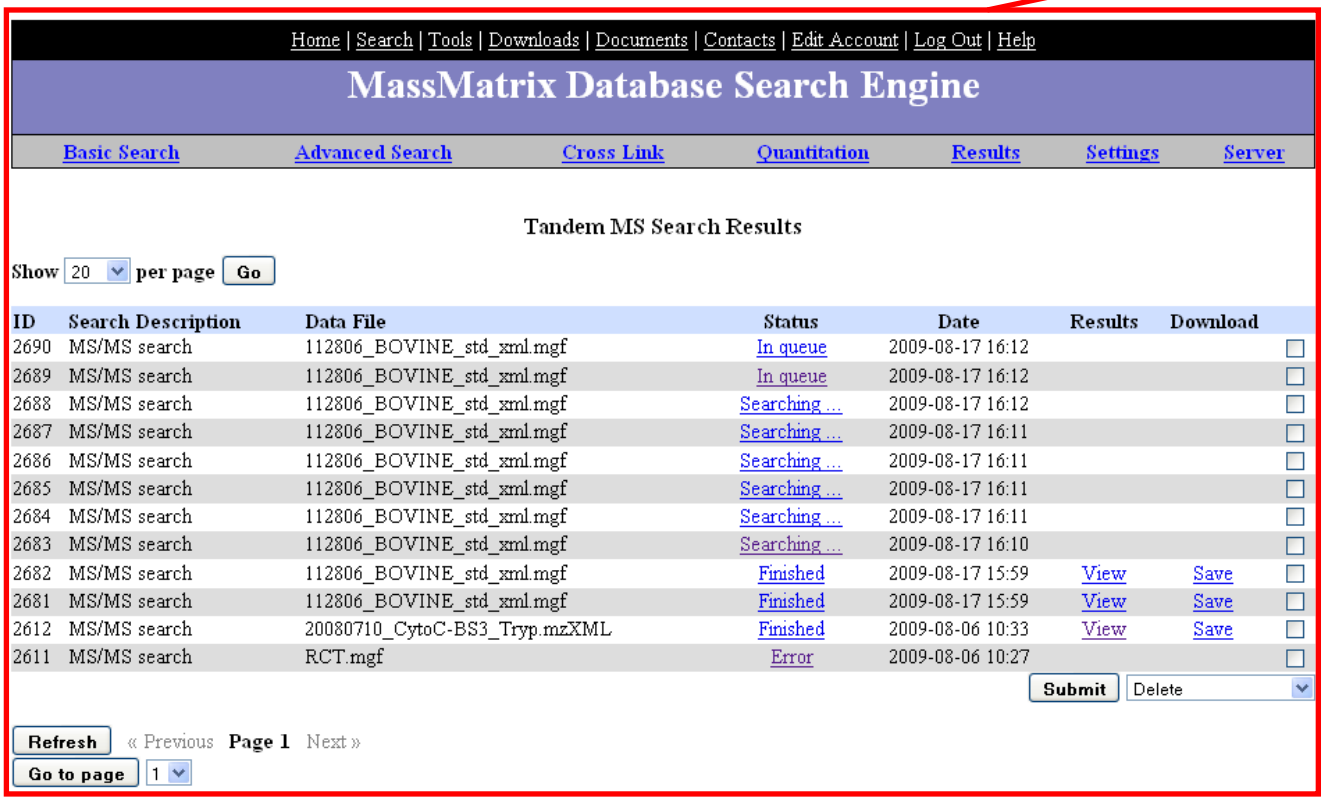
Step 3: Click "Search" button to submit the search.



You will be directed to a confirmation page if your submission is successful.

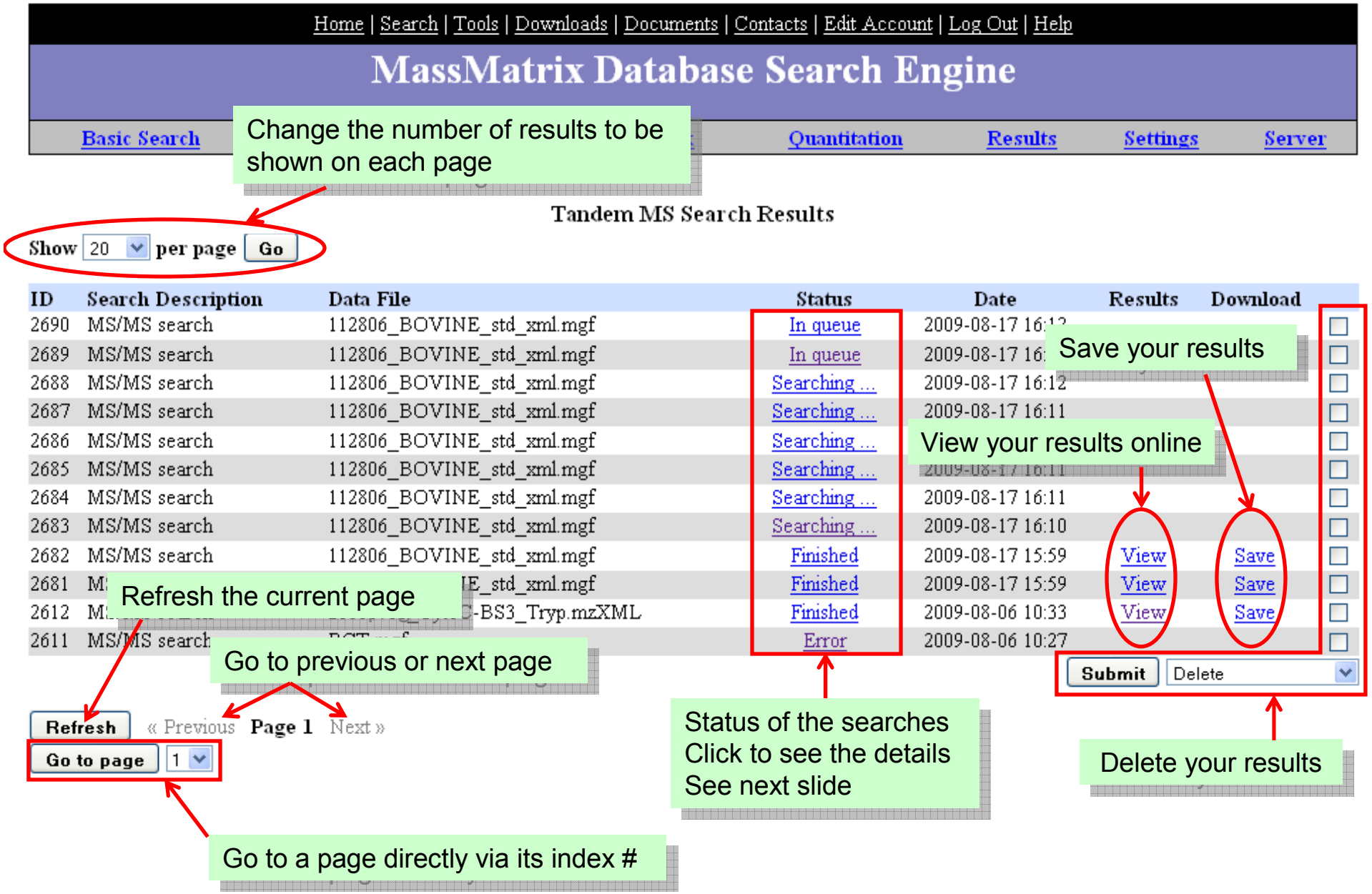
MassMatrix Search Engine – Results



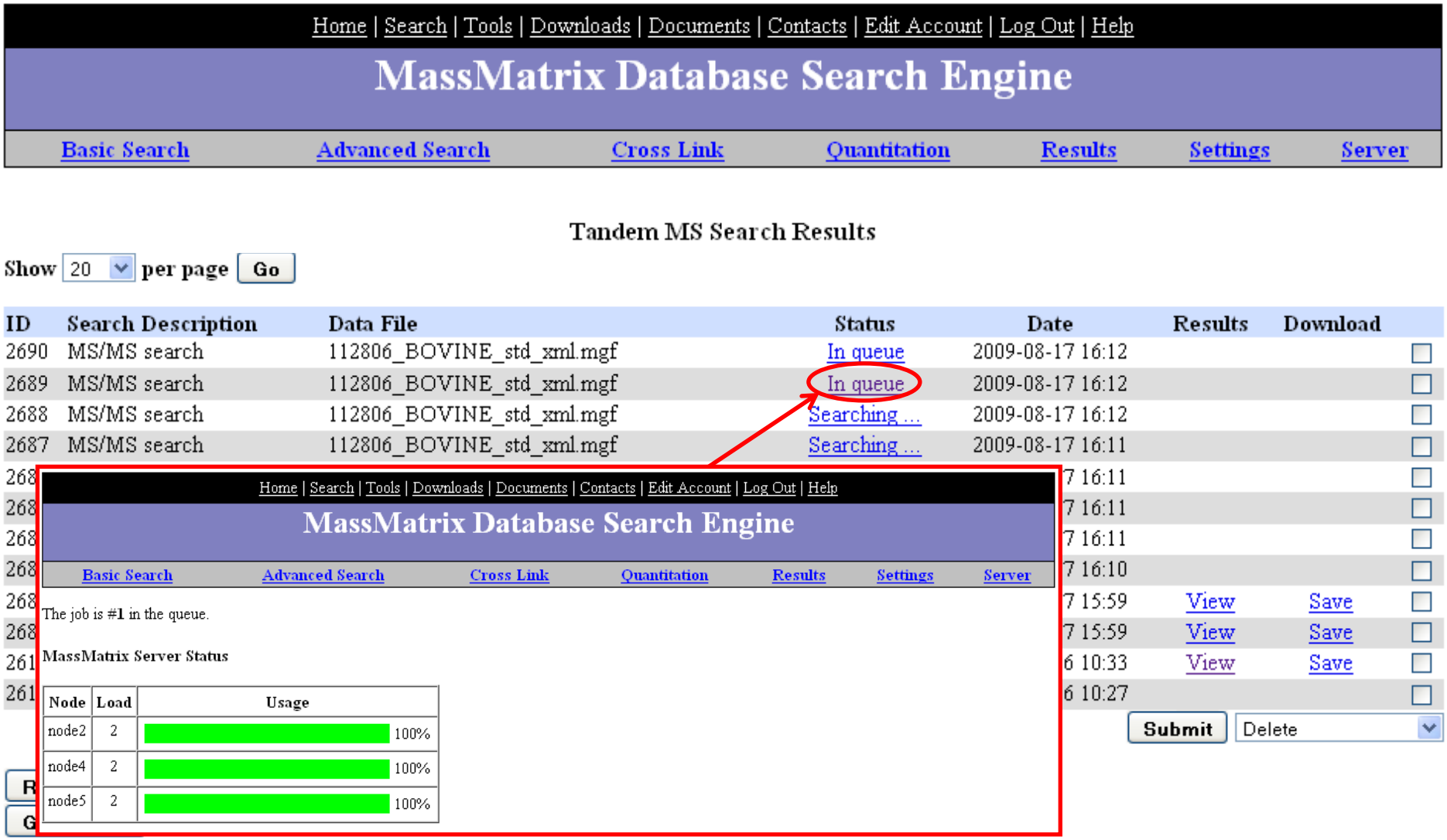


Click "**Results**" on the navigation bar for search to go to the results page. The results page lists all the search results that you have.

MassMatrix Search Engine – Results (Con't)

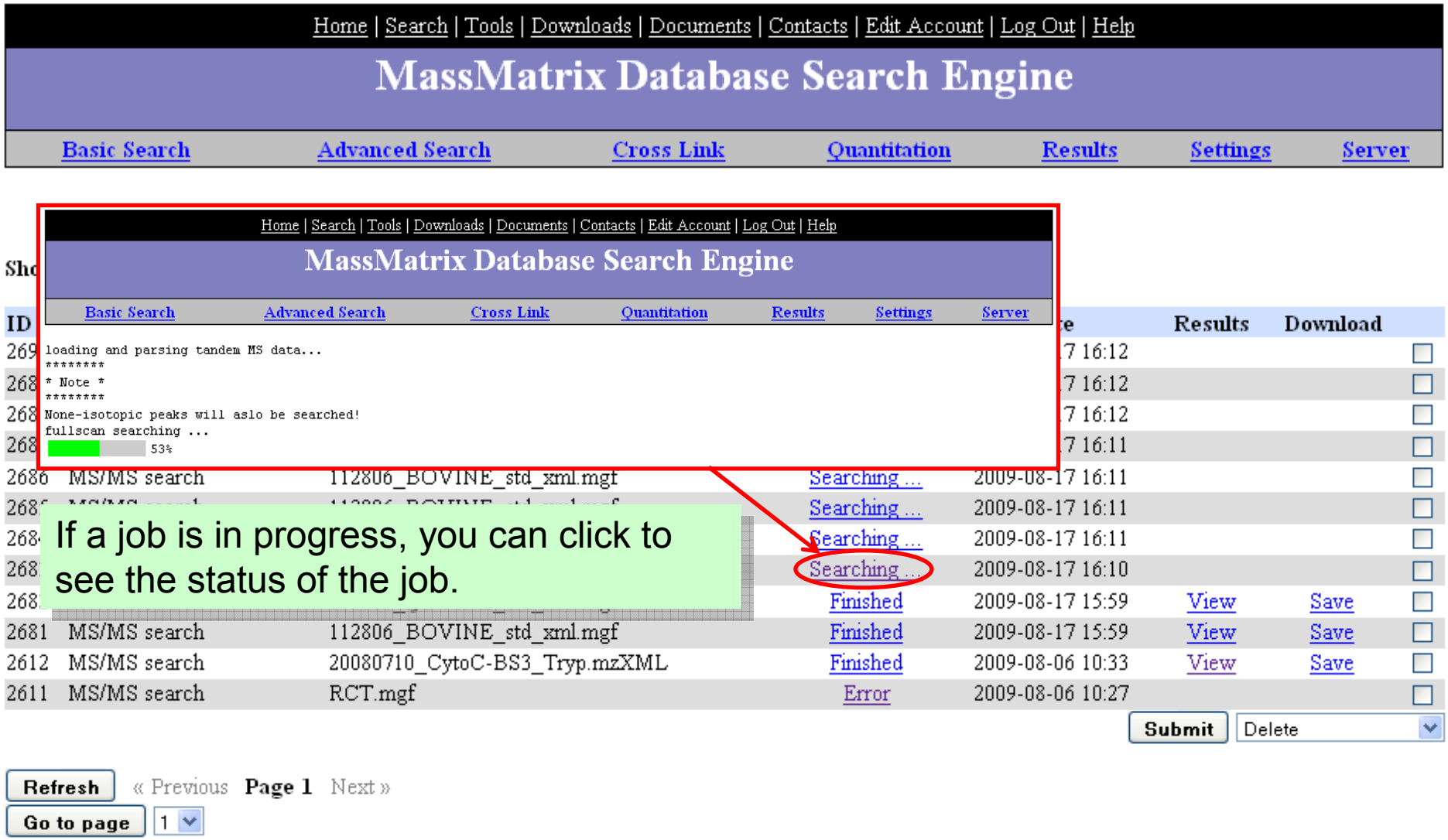


MassMatrix Search Engine – Results (Con't)

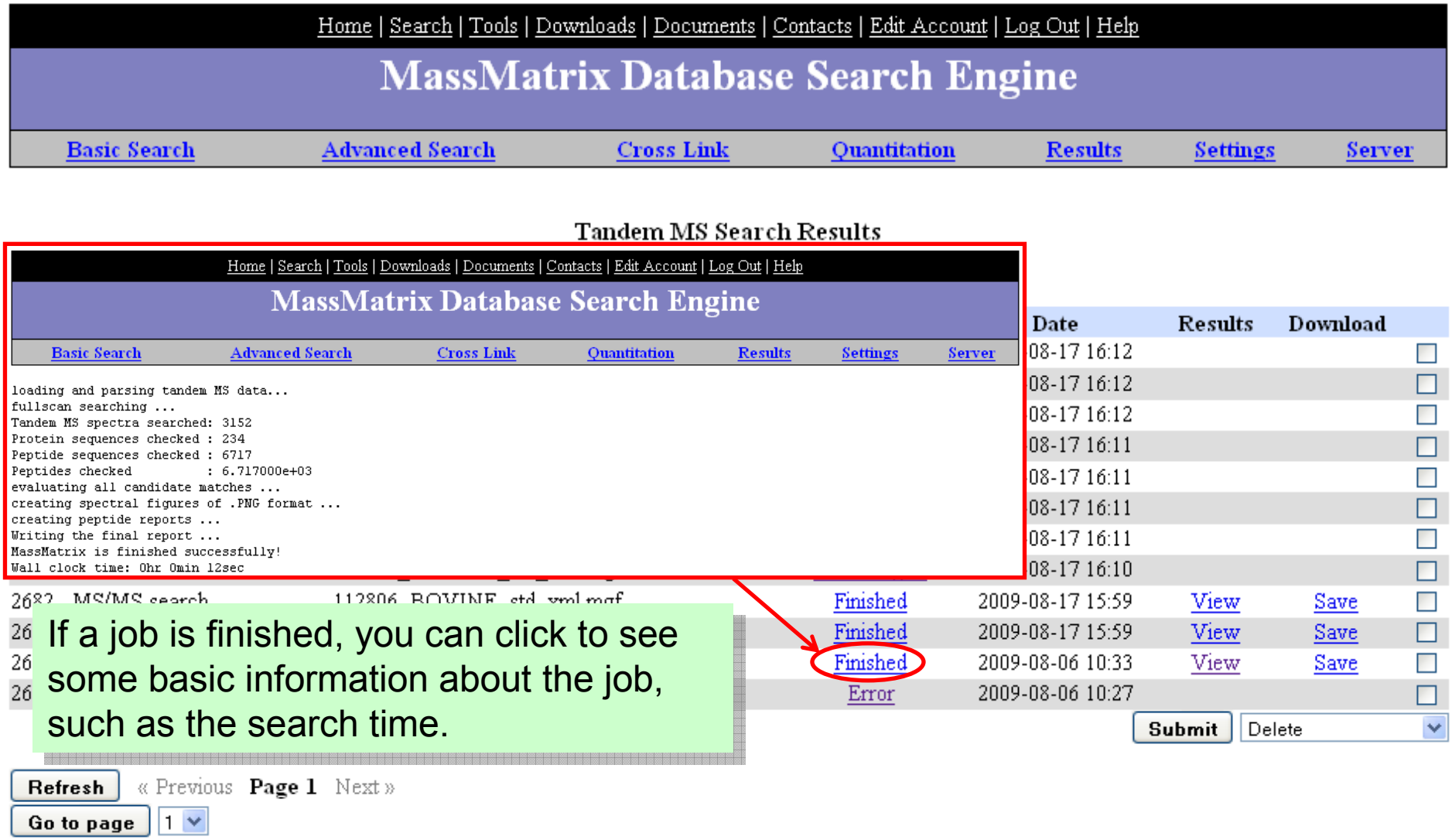


If a job is in queue, you can click to see its position in the queue.

MassMatrix Search Engine – Results (Con't)

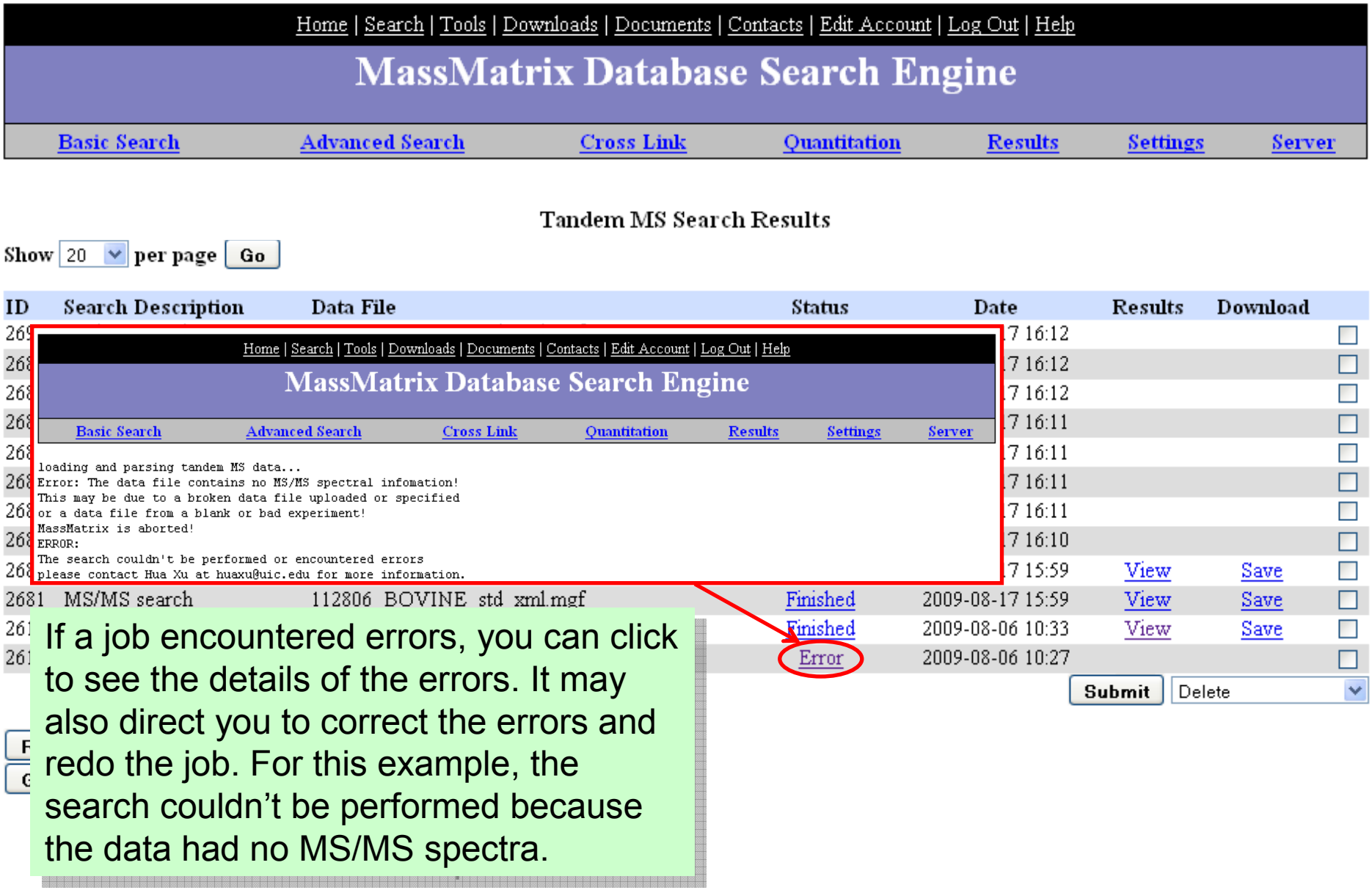


MassMatrix Search Engine – Results (Con't)



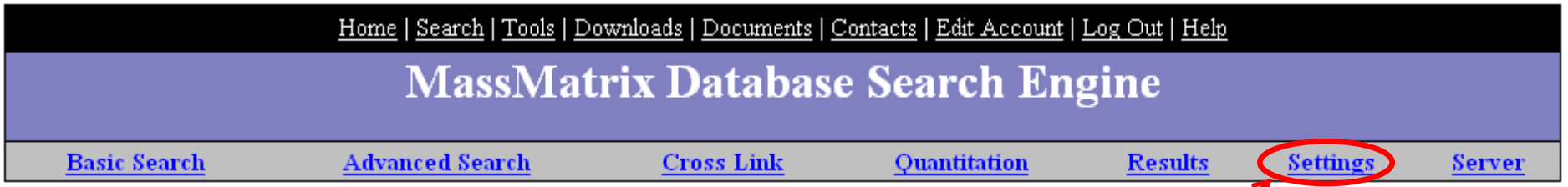
If a job is finished, you can click to see some basic information about the job, such as the search time.

MassMatrix Search Engine – Results (Con't)

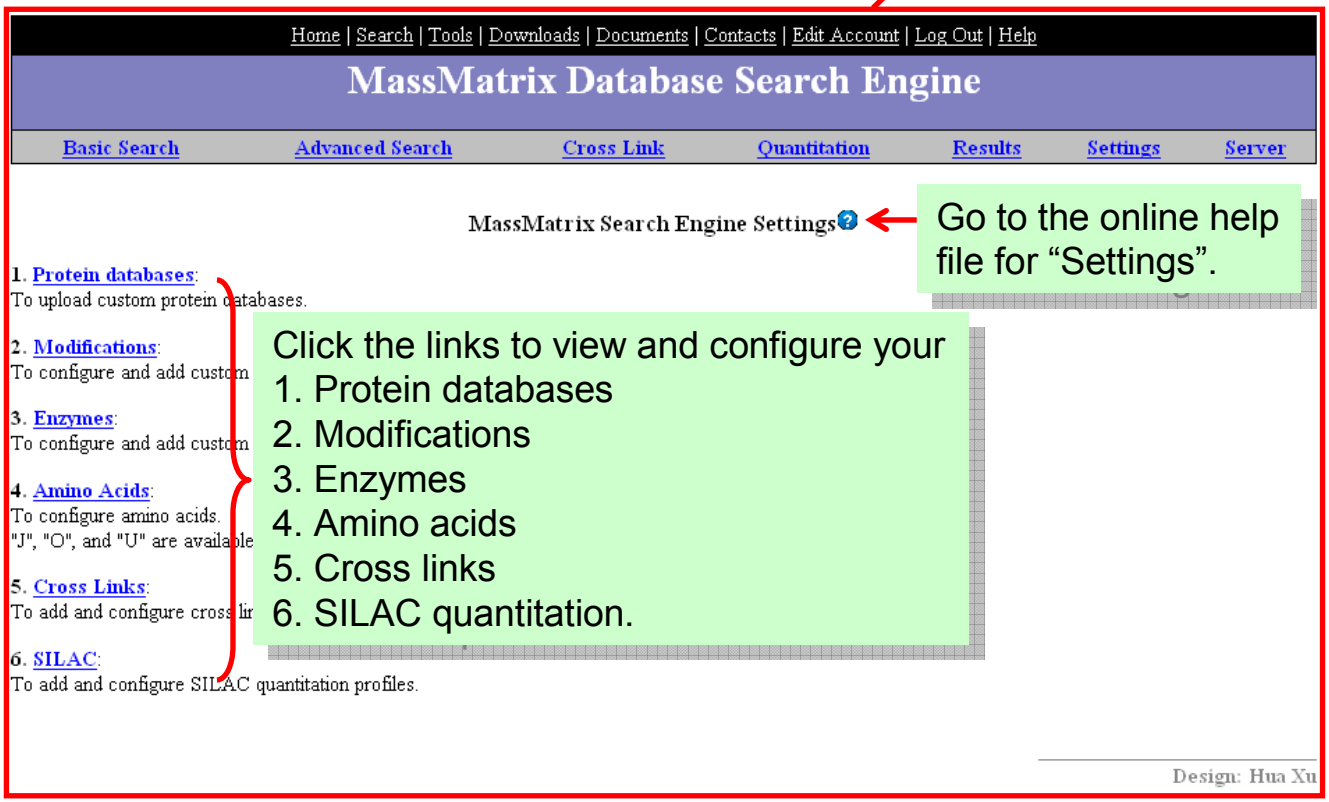


If a job encountered errors, you can click to see the details of the errors. It may also direct you to correct the errors and redo the job. For this example, the search couldn't be performed because the data had no MS/MS spectra.

MassMatrix Search Engine – Settings



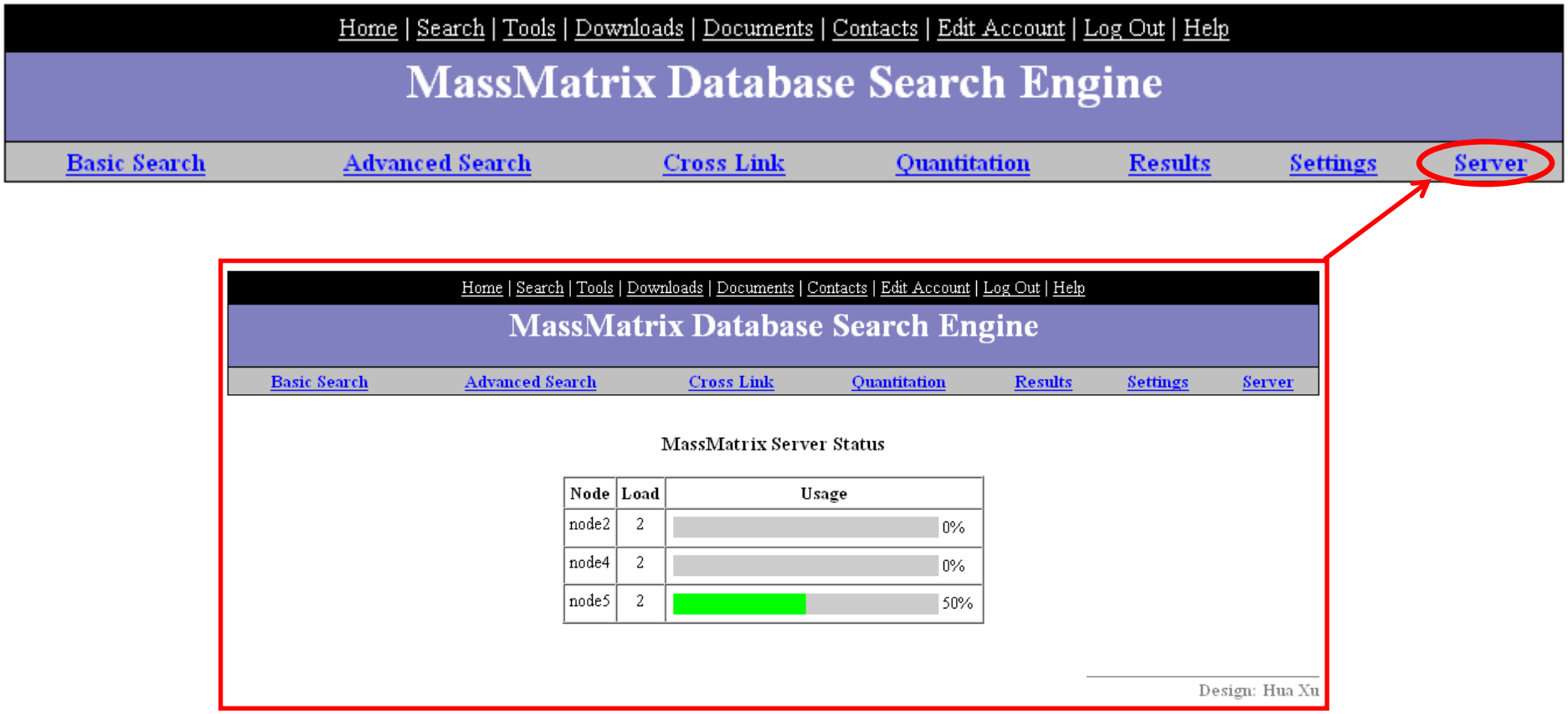
Click "**Settings**" on the navigation bar for search to go to the settings page. This page allows you to upload your own databases and configure the search engine.



For more details about MassMatrix Settings, please refer to

https://sourceforge.net/projects/massmatrix/files/MassMatrix_Manuals/MassMatrix%20Server%20Settings.pdf/download

MassMatrix Search Engine – Server



Click "**Sever**" on the navigation bar for search to go to the server status page. The server status page shows the usage of each compute node available on the server.

4. MassMatrix Search Form Help

1. Data files:
This field specifies the MS/MS data files that you want to search.
1) Click "Browse..."
2) Select a MS/MS data file to search. The data file should be stored locally on your own computer.
Multiple data files may be selected and searched at once by repeating the above steps.
Currently, mzXML, mzData and MGF file formats are supported in MassMatrix. File formats can be mixed for a single search.
Note:
For Thermo Fisher Scientific mass spectrometers, LCQ, LTQ, LTQ-FT ICR and LTQ-Orbitrap, to convert .RAW files to .mzXML or .mgf files, you may download MassMatrix file conversion tools by going to Downloads->MM File Conversion Tools.
Tip:
You can zip one or more MS/MS data files to a single zip file and use the zip file for search. Zipped file is of smaller size and it takes less time to upload the file to the server for search. Furthermore, choosing a zip file containing more than one MS/MS data files is equivalent to choosing the multiple original MS/MS data files.

2. Search option for multiple MS/MS files:
This field specifies the search option when you select multiple MS/MS data files to search.
If you choose "Individually", all MS/MS data files will be searched independently.
If you choose "Collectively", all MS/MS data files will be merged to a bigger MS/MS file and searched as a whole.
If you choose "Individually & Collectively", all MS/MS data files will be searched independently first, then be merged to a bigger MS/MS file and searched as a whole.

Note:
If you choose "Collectively" or "Individually & Collectively", please make sure that all the data files are of the same format (mzXML, mzData or MGF). If zip files uploaded, make sure that the original files are of the same format too (mzXML, mzData or MGF). Otherwise, the collective search cannot be performed.

3. Protein database to use:
This field specifies the protein database that you want to use.
Only one database can be selected.
You can upload your own databases by going to Settings->Protein databases.

4. Decoy database:
This field specifies the type of decoy database that you want to use.
If you select "Reversed", a protein database of the reversed protein sequences of the target database that you choose in "Database" field will be appended to the target database during searching.
If you select "Randomized", a protein database of the randomly reshuffled protein sequences of the target database that you choose in "Database" field will be appended to the target database during searching.
If you select "None", no decoy database will be used.
Decoy databases can be used to evaluate false positive discovery rate.

5. Enzyme to use:
This field specifies the enzymes used for digestion during sample preparation.
Multiple enzymes can be selected if you use a combination of enzymes during sample preparation.
In addition to the built-in enzymes, you can create you own enzymes by going to Settings->Enzymes.

Note: The special enzyme of "Nonspecific/Non-restricted" specifies non-restricted cleavage to use.

6. Missed Cleavages:
This field specifies the maximum number of missed cleavages allowed during proteolytic digestion.
Please specifies a number of 1 or 2 for your search to allow incomplete digestion that may occur.
If you are confident that your digestion goes to completeness, a number of 0 can also be chosen to get optimal results.
A large number specified in this field will increase the search space and search time exponentially and cause high false discovery rate.
Therefore, a large number is not recommended unless you think it is necessary.

7. Variable modifications:
This field specifies the modifications that may or may not modify the occurrences of certain amino acid residues.
Variable modifications add complexity as there are a great number of permutations of variably modified peptides for each sequence.
It will increase the search space. Therefore, please only choose necessary modifications for a large protein database.
Note:
You can create your own modifications by going to Settings->Modifications.

8. Fixed modifications:
This field specifies the modifications that modify all occurrences of certain amino acid residues.
Fixed modifications do not add complexity to the search. However, peptides with those amino acid residues that are incompletely modified will not be searched. Therefore, please choose fixed modification wisely and be sure that the modification can modify all occurrences of the specified amino acid residues.
Note:
You can create your own modifiactions by going to Settings->Modifications.

9. Mass spectrometer:
This field is only available in the basic search form. It specifies the mass spectrometer that you use.
All other advanced parameters will be set automatically according to your selected mass spectrometer and experiment type.

10. Experiment:
This field is only available in the basic search form. It specifies the type of your experiment: protein ID, protein characterization or disulfide search.
All other advanced parameters will be set automatically in accordance with your selected mass spectrometer and experiment type.

11. Precursor ion tolerance:
This field specifies the error tolerance for precursor peptide ion m/z values. The unit can be Da or ppm
The error tolerance should be specified according to the mass spectrometer that you use.
Typical settings for some common mass spectrometers are as follows.
LTQ-Orbitrap: 5-20 ppm

LTQ-FT ICR: 5-20 ppm
LTQ: 1.5-3.0 Da
LCQ: 1.5-3.0 Da

12. Product ion tolerance:
This field specifies the error tolerance for fragmented product ion m/z values. The unit is fixed to be Da. The error tolerance should be specified according to the mass spectrometer that you use.
Typical settings for some common mass spectrometers are as follows.
LTQ-Orbitrap: 0.5-0.8 Da for normal mode, 0.01-0.02 Da for Orbitrap-Orbitrap mode
LTQ-FT ICR: 0.5-0.8 Da
LTQ: 0.5-0.8 Da
LCQ: 0.5-0.8 Da

13. Max # PTM per peptide:
This field specifies the maximum number of variable modifications allowed for each peptide sequence. Due to the fact that variable modifications can dramatically increases the search space, search speed can be extremely slow and false positives can be severe. In order to limit the search space to get optimal results, a limited number of variable modifications should be allowed for each peptide sequence. However, if you are confident that you may have some peptides with a large number of variable modifications, please choose a proper big number. However, please be aware that the search many take a long time for a very large database with many variable modifications.

14. Mass type:
This field specifies the type of mass for precursor and product ions used during searching.

The monoisotopic or average mass for an ion can be specified.
It is recommended that monoisotopic mass is used for all types of searches and all types of mass spectrometers. Choosing "average" for a high mass accuracy mass spectrometer will cause erroneous results.

15. Min/Max peptide length:
These two fields specify the length of peptides to be searched.
A minimum length < 6 may cause many false positive peptide matches with small length.
A minimum length > 8 may cause the loss of true peptide matches with length < 8.
Therefore, it is recommended that a number between 6 and 8 be used.
The maximum length of peptides should be limited when several variable modifications are selected in order to make the search speed reasonably fast. This is due to the fact that long peptides tend to have more permutations of modification sites than short peptides. However a too small maximum length could cause the loss of long peptides. Typical settings of max peptide length for some common mass spectrometers are as follows.
LTQ-Orbitrap: 40-60
LTQ-FT ICR: 40-60
LTQ: 30-50 Da
LCQ: 30-40 Da

16. Min pp, pp_{tag} scores of peptides for output:
The quality of a peptide match is mainly evaluated by three statistical scores: pp, pp_2, pp_{tag}.
These fields specify the score thresholds for those three scores. The min pp score is the threshold for pp and pp_2 scores. The min pp_{tag} is the threshold for pp_{tag} score. A too low threshold setting will cause many peptide

matches with small scores and of low quality in your final results. A too high threshold setting may cause the loss of peptide matches of good quality.
For normal protein identification, a setting of 4.0-6.0 can be used for min pp score and a setting of 1.0-2.3 can be used for min pp_{tag}.
A low setting of those two thresholds can be used when you want all possible peptide matches output in your results. For example, when you perform a search of peptides and proteins with intact disulfide bonds or cross links against a limited protein database, a threshold as low as 0.1 for min pp score and 0.01 for min pp_{tag} may be specified to allow all possible peptide matches with cross links in your final results. This may be necessary when pp, pp_2, pp_{tag} scores for big peptides with disulfide bonds or cross links are very low due to the fact the MS/MS spectra of those peptides have many product ions and there are many different peptides having similar MS/MS spectra.

17. Max # match/spec:
This field specifies the maximum number of candidate peptide matches for each spectrum output in the result.
Under some circumstances, a spectrum may have multiple candidate peptide matches with close statistical scores. MassMatrix will output up to "max # match/spec" number of those matches with top scores. A setting bigger than 1 will allow you to evaluate the other competing peptide matches besides the one with the best scores.

18. Max # comb/match:
This field specifies the maximum number of combination of different modification sites for a peptide match with modifications output in the result.

Under many circumstances, peptides with the same sequence and the same set of modifications, but different specific modification sites will have very close statistical scores. MassMatrix will output up to "max # com/match" number of them. A setting bigger that 1 is necessary under most cases when modification sites need to be determined.

19. Fragmentation method:
This field specifies the fragmentation method used during mass spectrometry to MS/MS spectra. CID, ETD, ECT are supported.
Note:
Performance of MassMatrix on ECD data has not been tested.

20. C13 isotope ions:
This field specifies whether or not non-monoisotopic peptide ions be searched.
For high mass accuracy machines, peptide ions with C13 isotopes (non-monoisotopic ions) may undergo fragmentation to create MS/MS spectra. Therefore, it is necessary to choose "yes" to get optimal results. A setting of "Auto" is always recommended, by which MassMatrix will determine the best option for you.

21. Cross link:
This field specifies the intact cross links you want to search.
You can create your own cross links to search by going to Settings->Cross Links.
Note:
In order to search peptides with disulfides or cross links, you also have to choose a proper search mode in the field of "Cross link mode". By default, the search mode of disulfides or cross links is "disabled", which means MassMatrix will not try to search any peptides with disulfides or cross links. Please refer to Cross link

mode for more details.

22. Cross link mode:
This field specifies the search mode for peptides with disulfide or cross links.
"Disabled":
No search of peptides with disulfide or cross links will be performed.
"Exploratory":
In the exploratory search mode, all possible cross link site residues in the protein sequences are considered to be variable cross link sites, i.e. all site residues may or may not form cross links. During searching, MassMatrix will generate all possible combinations of cross links by assuming that any two site residues are capable of forming a cross link. Consider a protein with n cysteine residues. During exploratory search mode of disulfide bonds, MassMatrix will generate n(n-1)/2 possible combinations of single disulfide bond for the protein (n= number of cysteine residues).
"Confirmatory":
In the confirmatory search mode, only the cross links specified in the protein database will be considered and searched against experimental data. Cross links are specified in the sequence by uploading your custom database. In the special .fasta protein databases or .bas MassMatrix databases, cross links are coded as "($*i*)" where X is the site residue (e.g. C for disulfide bonds), *i* is the index number of the specified cross link. Each cross link has two related cross link site residues with the same label of "($*i*)"
For example, in the confirmatory search of disulfide bonds against a protein database containing the following sequence

```
>Ribonuclease A from bovine pancreas
```

```
KETAAAKFER       10
QHMDSSTSAA       20
SSSNYC($1)NQMM   30
KSRNLTKDRC($2)   40
KPVNTFVHES       50
LADVQAVC($3)SQ   60
KNVAC($4)KNGQT   70
NC($4)YQSYSTMS   80
ITDC($1)RETGSS   90
KYPNC($2)AYKTT   100
QANKHIIVAC($3)   110
EGNPYVPVHF       120
DASV             130
```

only four native disulfide bond in the protein labeld by "($1)", "($2)", "($3)", and "($4)" will be searched.

"Semi-exploratory":

In the semi-exploratory mode, an exploratory search will be performed. However, the search of cross links will be limited to those site residues with a label of "($)" or ($*i*)" where *i* can be any number.

For example, in the semi-exploratory search of disulfide bonds against a protein database containing the following sequence

```
>Ribonuclease A from bovine pancreas
KETAAAKFER       10
QHMDSSTSAA       20
```

```
SSSNYC($1)NQMM   30
KSRNLTKDRC($2)   40
KPVNTFVHES       50
LADVQAVCSQ       60
KNVACKNGQT       70
NCYQSYSTMS       80
ITDC($1)RETGSS   90
KYPNC($2)AYKTT  100
QANKHIIVAC      110
EGNPYVPVHF      120
DASV            130
```

only disulfide bonds between the four Cys with a label of "($1)" or "($2)", i.e. 4*(4-1)/2 = 6 disulfide bonds, will be considered.

23. Cross link sites cleavability:

This field specifies whether the cross link sites are cleavable by the specified enzyme(s) or not. The default setting is "Non applicable", which means that the cross link sites are not among the cleavage sites of the specified enzyme(s). If the cross link sites are among the cleavage sites of the specified enzyme(s), you will have to specify this field. For example, the cross link sites are lysine rediues and the specified enzyme is trypsin. If you choose "Non-cleavable by enzyme", the lysine residues that are cross linked with another lysine will not be cleaved by enzyme during searching. If you choose "Cleavable by enzyme", the lysine residues that are cross linked with another lysine will also be cleaved by enzyme like normal lysine residues during searching.

24. Max # cross links/peptide:
This field specifies the maximum number of cross links allowed for each peptide. Only 1 and 2 can be choosen. If 1 is chosen, peptides with up to 1 cross links will be searched. If 2 is selected, peptides with up to 2 cross links will be searched.

25. How to search inter-protein cross-links:
In order to search inter-protein cross-links between different proteins or inter-chain cross-links for a protein with multiple chains (such as Insulin), all the protein sequences and sequences for different chains have to be included as one protein in the .FASTA or .BAS database. Different proteins and chains have to be on different rows and start with "~".
For example, in order to search "K-K" cross-links between two proteins, Cytochrome C and Lysosome, a .FASTA protein database has to be constructed as follows:

```
>Cytochrome C and Lysosome
MGDVEKGKKIFVQKCAQCHTVEKGGKHKTGPNLHGLFGRKTGQAPGFTYTDANKNKGITWKEETLMEYLE
NPKKYIPGTKMIFAGIKKKTEREDLIAYLKKATNE
~MRSLLILVLCFLPLAALGKVFGRCELAAAMKRHGLDNYRGYSLGNWVCAAKFESNFNTQATNRNTDGSTD
YGILQINSRWWCNDGRTPGSRNLCNIPCSALLSSDITASVNCAKKIVSDGNGMNAWVAWRNRCKGTDVQA
WIRGCRL
```

In this way, MassMatrix will generate all peptides from both proteins with and without cross-links and also those due to inter-protein cross-links between Cytochrome C and Lysosome.

Another example is Insulin containing two chains linked by disulfide bonds. In order to search inter-chain

disulfide bonds for the protein, you have to construct a .FASTA or .BAS database as follows:

```
>Insulin
GIVEQC($1)C($2)ASVC($1)SLYQLENYC($3)N
~FVNQHLC($2)GSHLVEALYLVC($3)GERGFFYTPKA
```

Confirmatory disulfide search for Insuline can also be performed, since all native disulfide bonds are specified in the above database.

26. Quantitation:
This field specifies the method of quantitation that you want to use. Currently, quantitation by use of iTRAQ, TMT or ^{15}N labeling are supported. In the future, quantitation by use of SILAC and ^{18}O labeling will be supported.

27. Quantitation statistics:
This field specifies the statistical method for quantitation. Details of those methods are not covered in this help file. But the mathematical proofs and also the evaluation of those different methods will be published in a scientific journal. It is recommended that you always use the default method.

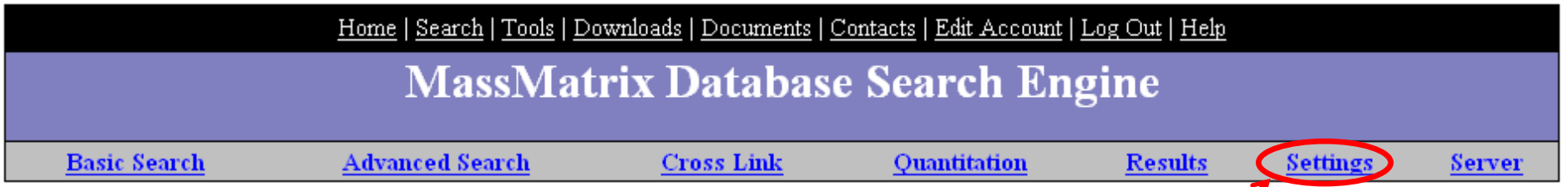
28. Comment:
This field allows you to give a title to your search so that you may recognize your search afterwards.

29. Expert Options:
This field is only used to enable un-published functions in MassMatrix. Un-published functions in MassMatrix are either not validated or confidential. So you may always leave it blank.

For more information, please contact **Hua Xu**.

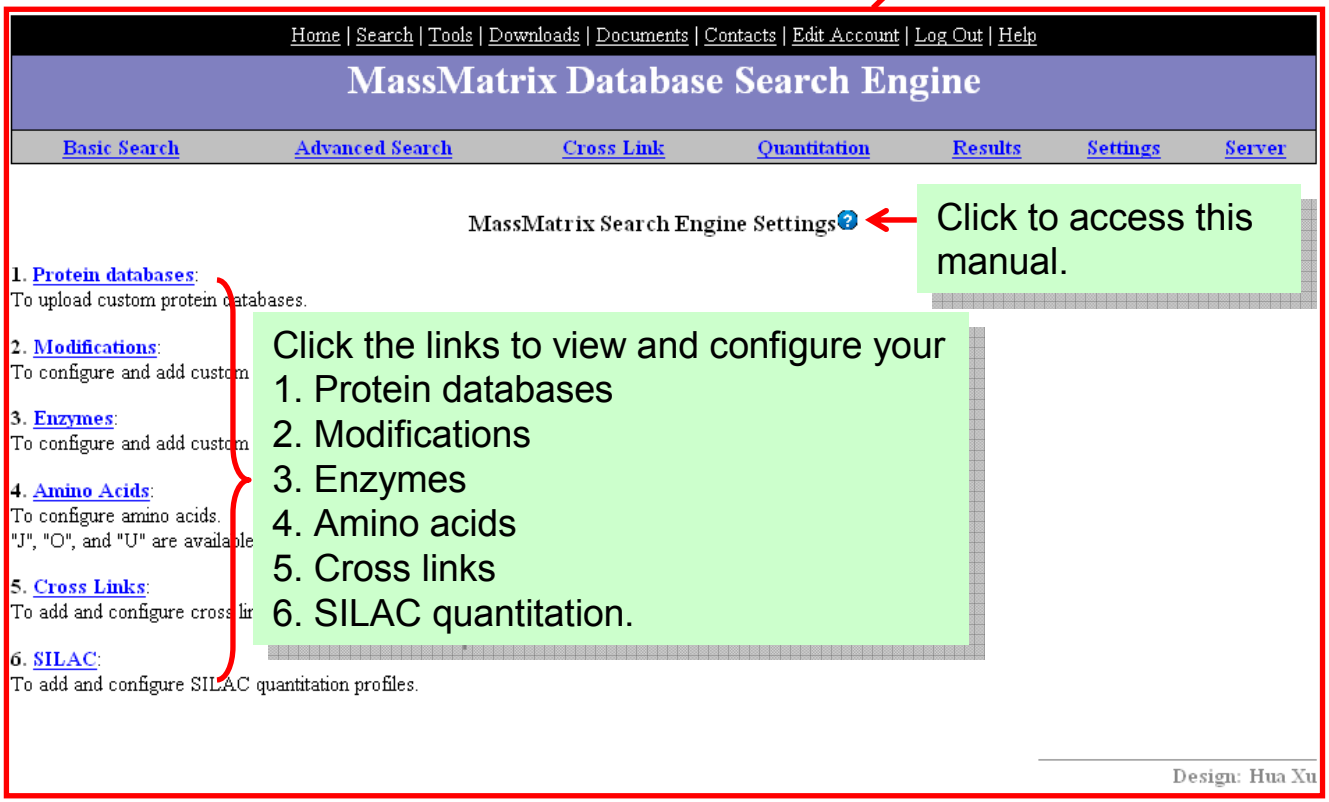
5. MassMatrix Server Settings

MassMatrix Server Settings

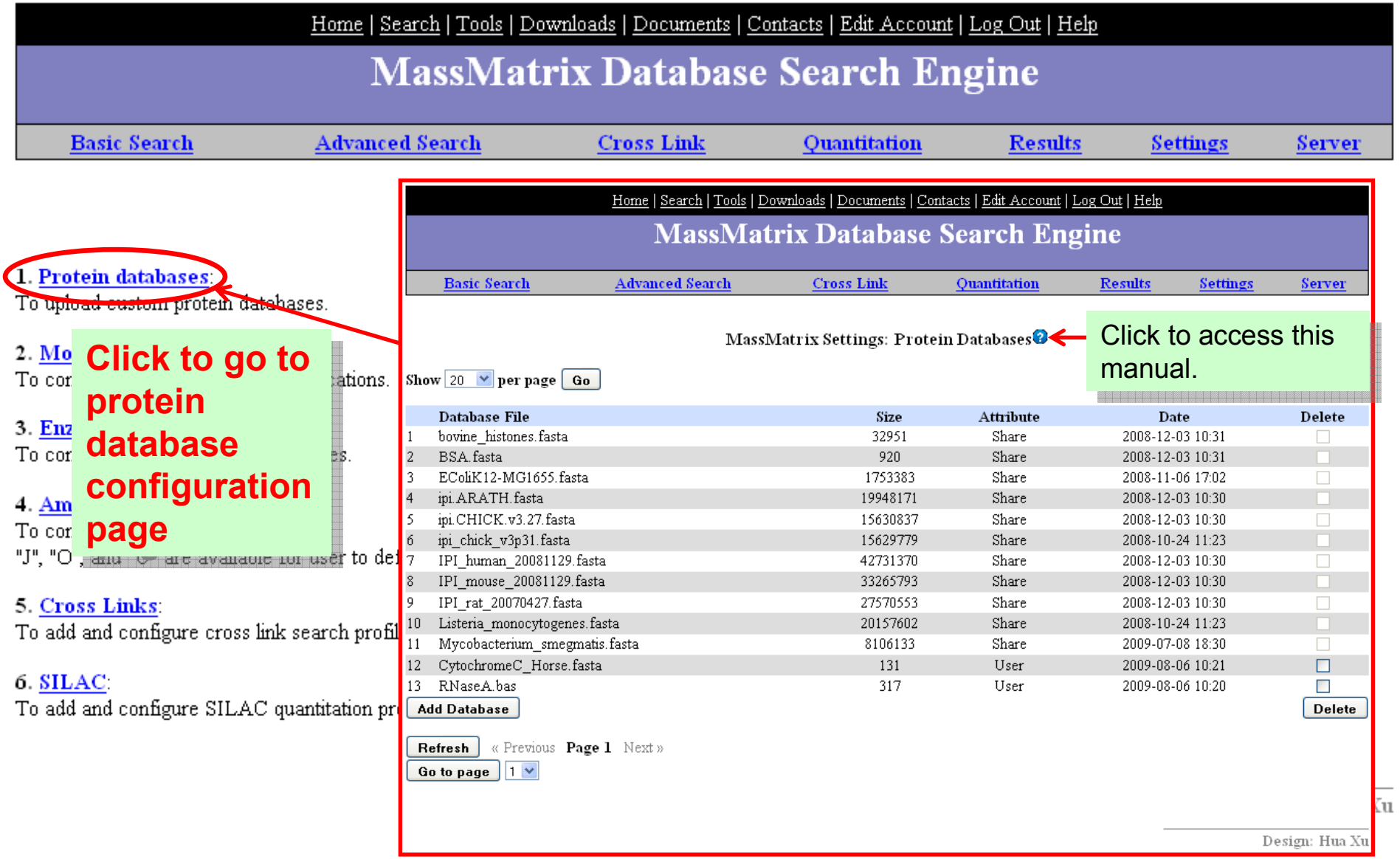


Log in the server and go the search engine.

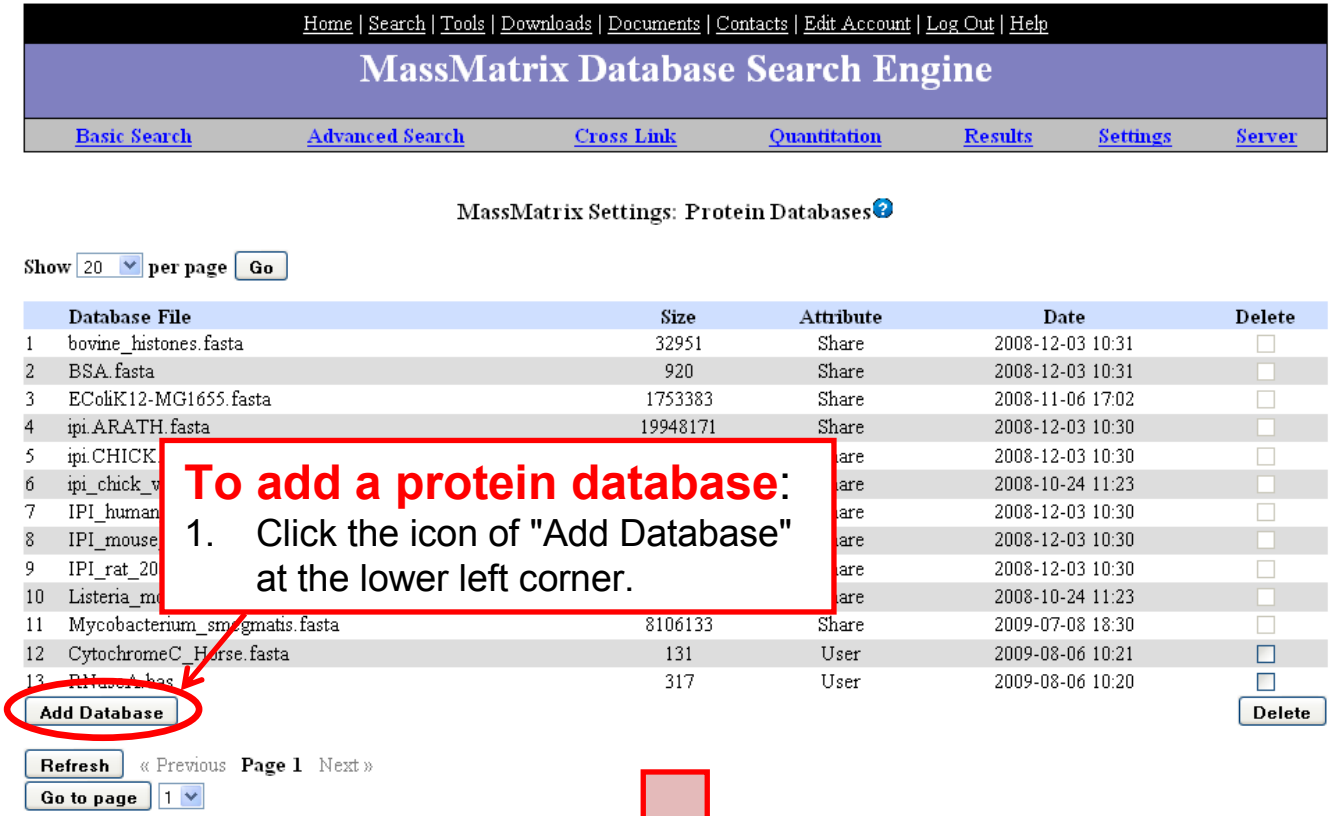
Click "**Settings**" on the navigation bar for search to go to the settings page.



MassMatrix Server Settings – Protein Databases

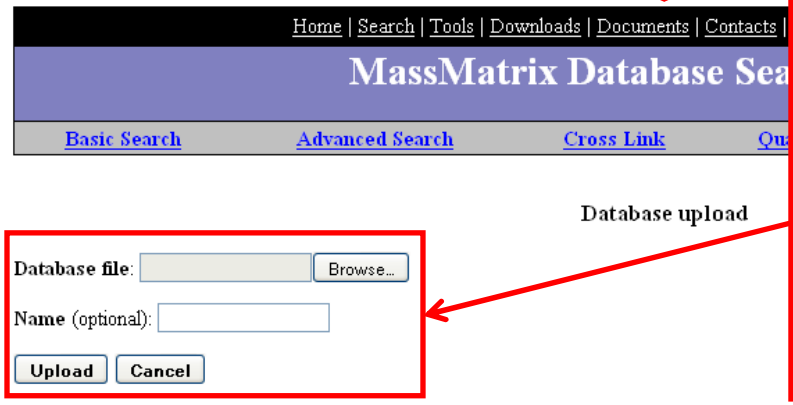


MassMatrix Server Settings – Protein Databases



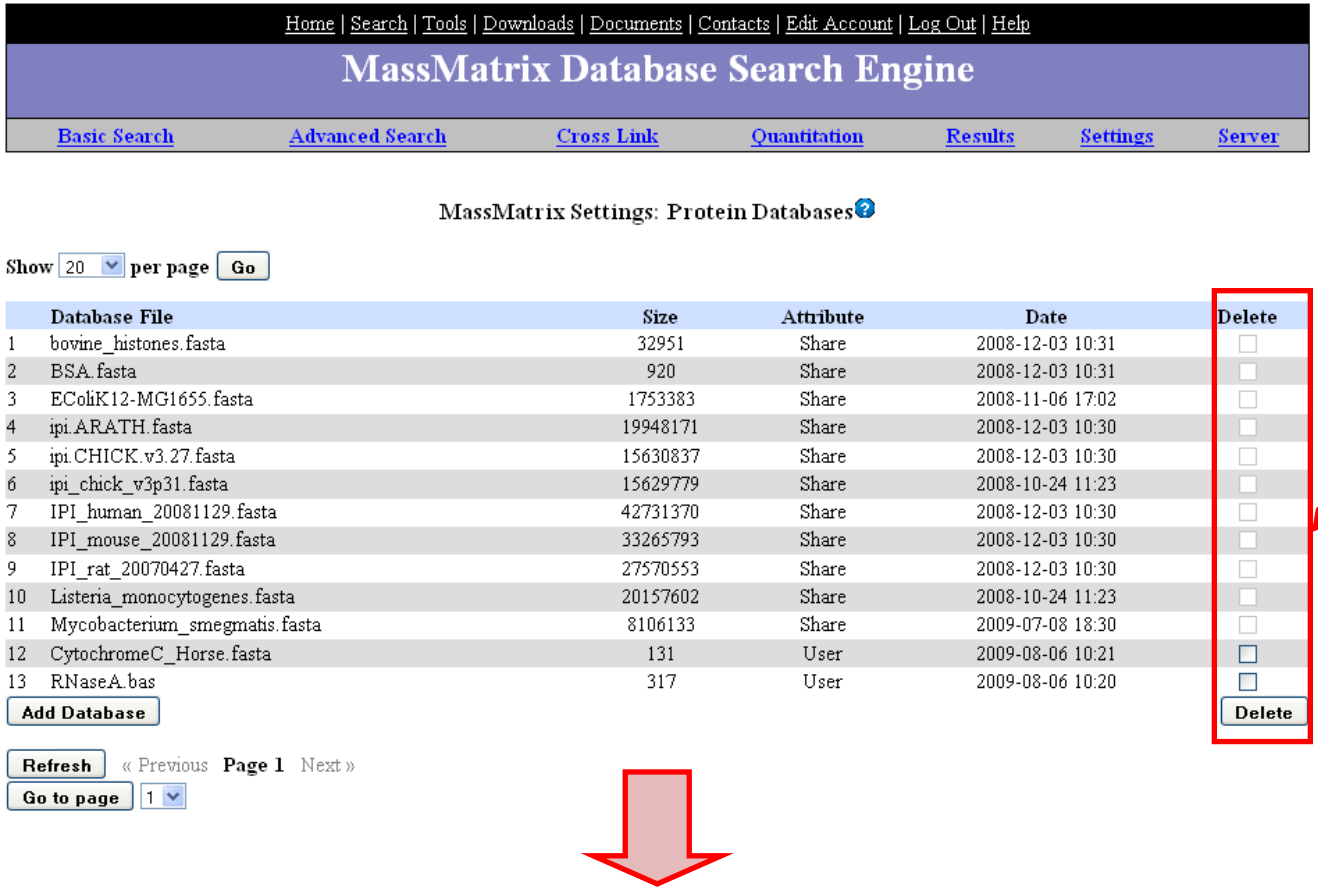
To add a protein database:

1. Click the icon of "Add Database" at the lower left corner.



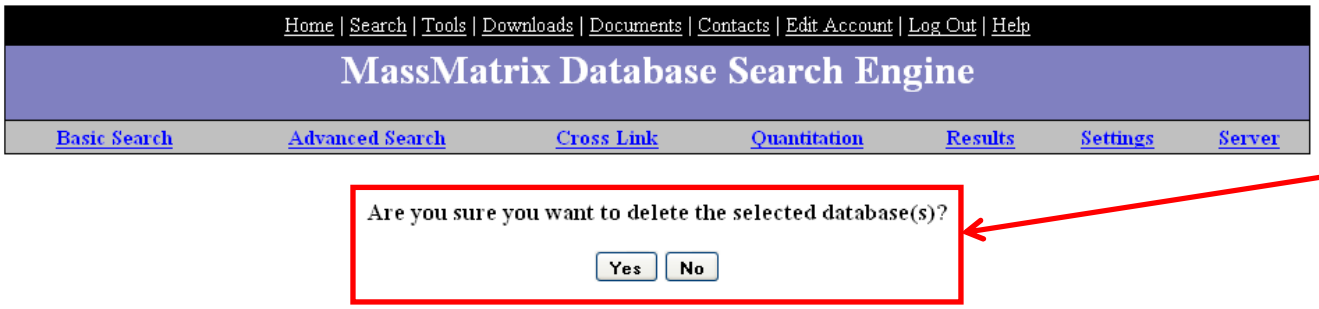
2. Click the icon of "Browse..".
3. Choose the database file that you want to upload in the pop-up window.
4. You may rename the file. Note that you have to give a proper extension name of the file. Currently, .fasta and .bas are supported.
5. Click the icon of "Upload" to upload the database file or click the icon of "Cancel" to cancel the upload.

MassMatrix Server Settings – Protein Databases



To delete protein databases:

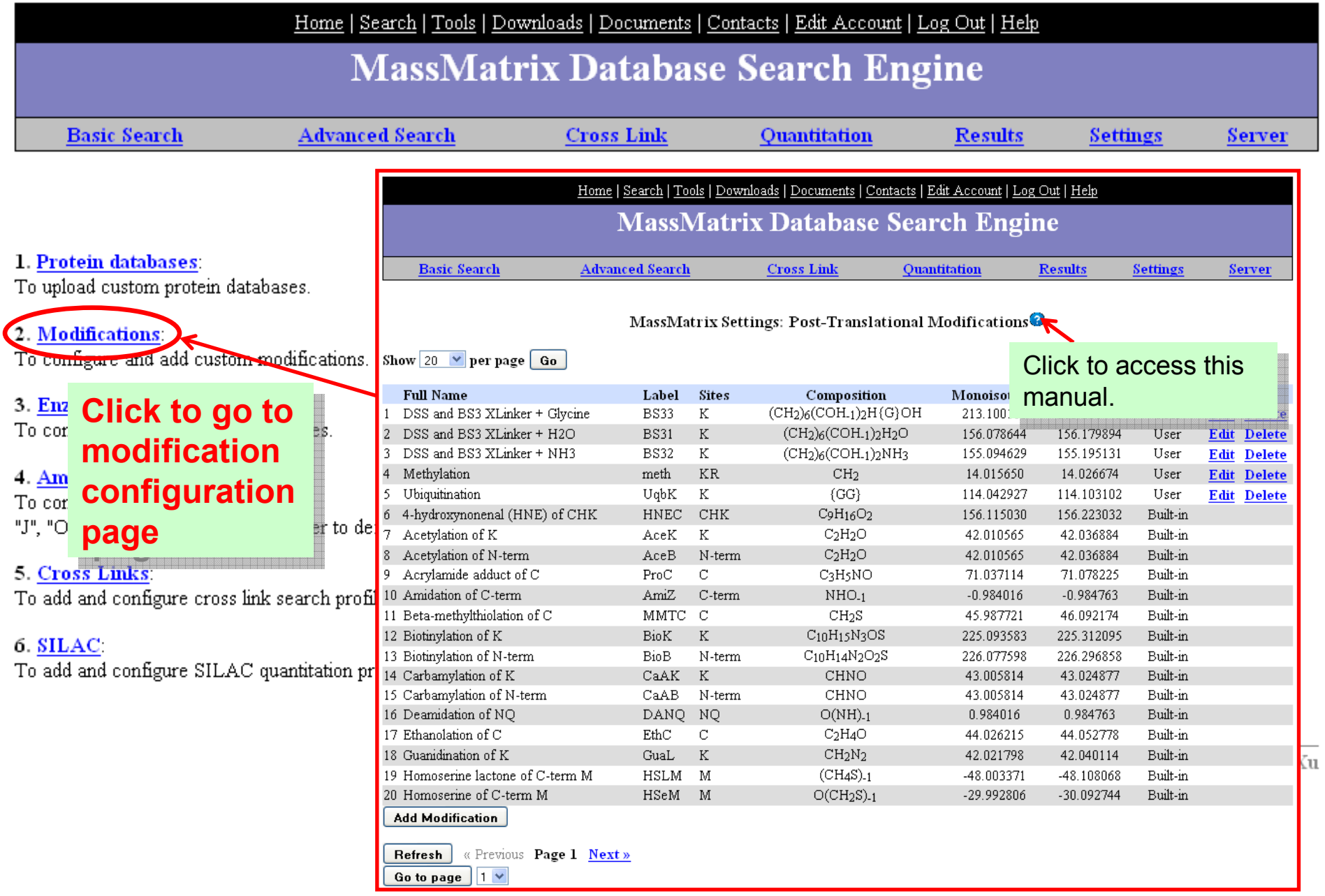
1. Select the boxes of the databases that you want to delete.
2. Click the icon of "Delete" at the lower right corner.



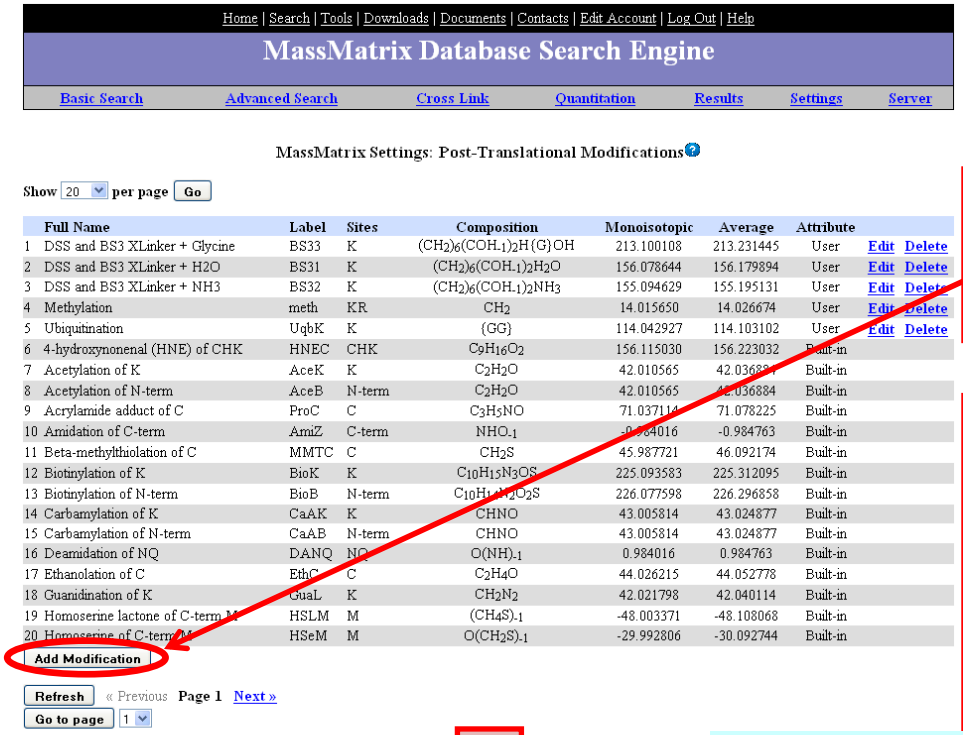
3. Confirm the deletion by clicking the icon of "Yes" or cancel the deletion by clicking the icon of "No".

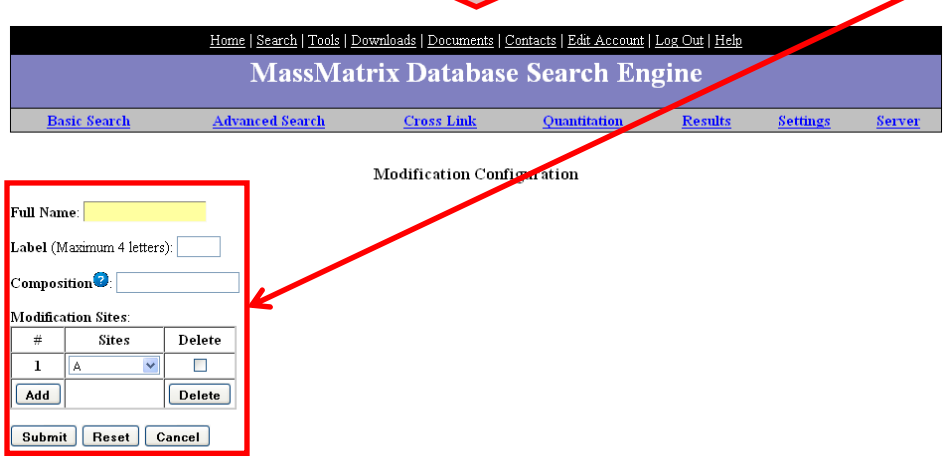
Note: Shared protein databases cannot be deleted.

MassMatrix Server Settings – Modifications



MassMatrix Server Settings – Modifications





To add a modification:

1. Click the icon of "Add Modification" at the lower left corner.
2. Specify the full name of your modification.
3. Specify the label of your modification. The label of the modification will be used in the result report.
4. Specify the chemical formula of the modification (click the link below for chemical formula syntax).

http://sourceforge.net/projects/massmatrix/files/MassMatrix_Manuals/Chemical%20Formula%20Syntax.pdf/download

Mass shift will be calculated automatically according to the chemical formula.

5. Specify the modification sites. Multiple sites may be specified. Sites can be added or deleted by the icons of "Add" and "Delete".
6. Click the icon of "Submit" to submit the changes or click the icon of "Cancel" to cancel the change.

MassMatrix Server Settings – Modifications

To edit a modification:

1. Click the "Edit" link of the modification that you want to modify.

2. Edit the full name of your modification.
3. Edit the label of your modification.
4. Edit the chemical formula of the modification.
5. Edit the modification sites. Multiple sites may be specified. Sites can be added or deleted by the icons of "Add" and "Delete".
6. Click the icon of "Submit" to submit the changes or click the icon of "Cancel" to cancel the change.

Note: Built-in modifications cannot be edited.

MassMatrix Server Settings – Modifications

	Full Name	Label	Sites	Composition	Monoisotopic	Average	Attribute	
1	DSS and BS3 XLinker + Glycine	BS33	K	$(CH_2)_6(COH_{-1})_2H\{G\}OH$	213.100108	213.231445	User	Edit Delete
2	DSS and BS3 XLinker + H2O	BS31	K	$(CH_2)_6(COH_{-1})_2H_2O$	156.078644	156.179894	User	Edit Delete
3	DSS and BS3 XLinker + NH3	BS32	K	$(CH_2)_6(COH_{-1})_2NH_3$	155.094629	155.195131	User	Edit Delete
4	Methylation	meth	KR	CH_2	14.015650	14.026674	User	Edit Delete
5	Ubiquitination	UqbK	K	{GG}	114.042927	114.103102	User	Edit Delete
6	4-hydroxynonenal (HNE) of CHK	HNEC	CHK	$C_9H_{16}O_2$	156.115030	156.223032	Built-in	
7	Acetylation of K	AceK	K	C_2H_2O	42.010565	42.036884	Built-in	
8	Acetylation of N-term	AceB	N-term	C_2H_2O	42.010565	42.036884	Built-in	
9	Acrylamide adduct of C	ProC	C	C_3H_5NO	71.037114	71.078225	Built-in	
10	Amidation of C-term	AmiZ	C-term	NHO_{-1}	-0.984016	-0.984763	Built-in	
11	Beta-methylthiolation of C	MMTC	C	CH_2S	45.987721	46.092174	Built-in	
12	Biotinylation of K	BioK	K	$C_{10}H_{15}N_3OS$	225.093583	225.312095	Built-in	
13	Biotinylation of N-term	BioB	N-term	$C_{10}H_{14}N_2O_2S$	226.077598	226.296858	Built-in	
14	Carbamylation of K	CaAK	K	CHNO	43.005814	43.024877	Built-in	
15	Carbamylation of N-term	CaAB	N-term	CHNO	43.005814	43.024877	Built-in	
16	Deamidation of NQ	DANQ	NQ	$O(NH)_{-1}$	0.984016	0.984763	Built-in	
17	Ethanolation of C	EthC	C	C_2H_4O	44.026215	44.052778	Built-in	
18	Guanidination of K	GuaL	K	CH_2N_2	42.021798	42.040114	Built-in	
19	Homoserine lactone of C-term M	HSLM	M	$(CH_4S)_{-1}$	-48.003371	-48.108068	Built-in	
20	Homoserine of C-term M	HSeM	M	$O(CH_2S)_{-1}$	-29.992806	-30.092744	Built-in	

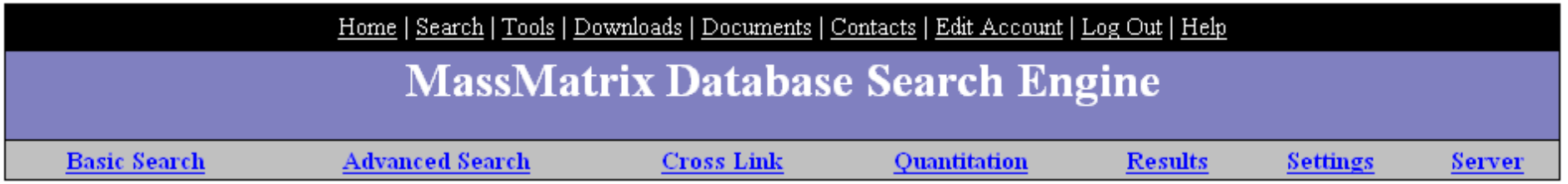
To delete a modification:

1. Click the “Delete” link of the modification that you want to delete.

2. Confirm the deletion by clicking the icon of "Yes" or cancel the deletion by clicking the icon of "No"

Note: Built-in modifications cannot be deleted.

MassMatrix Server Settings – Enzymes



1. **Protein databases**:
To upload custom protein databases.

2. **Modifications**:
To configure and add custom modifications.

3. **Enzymes**:
To configure and add custom enzymes.

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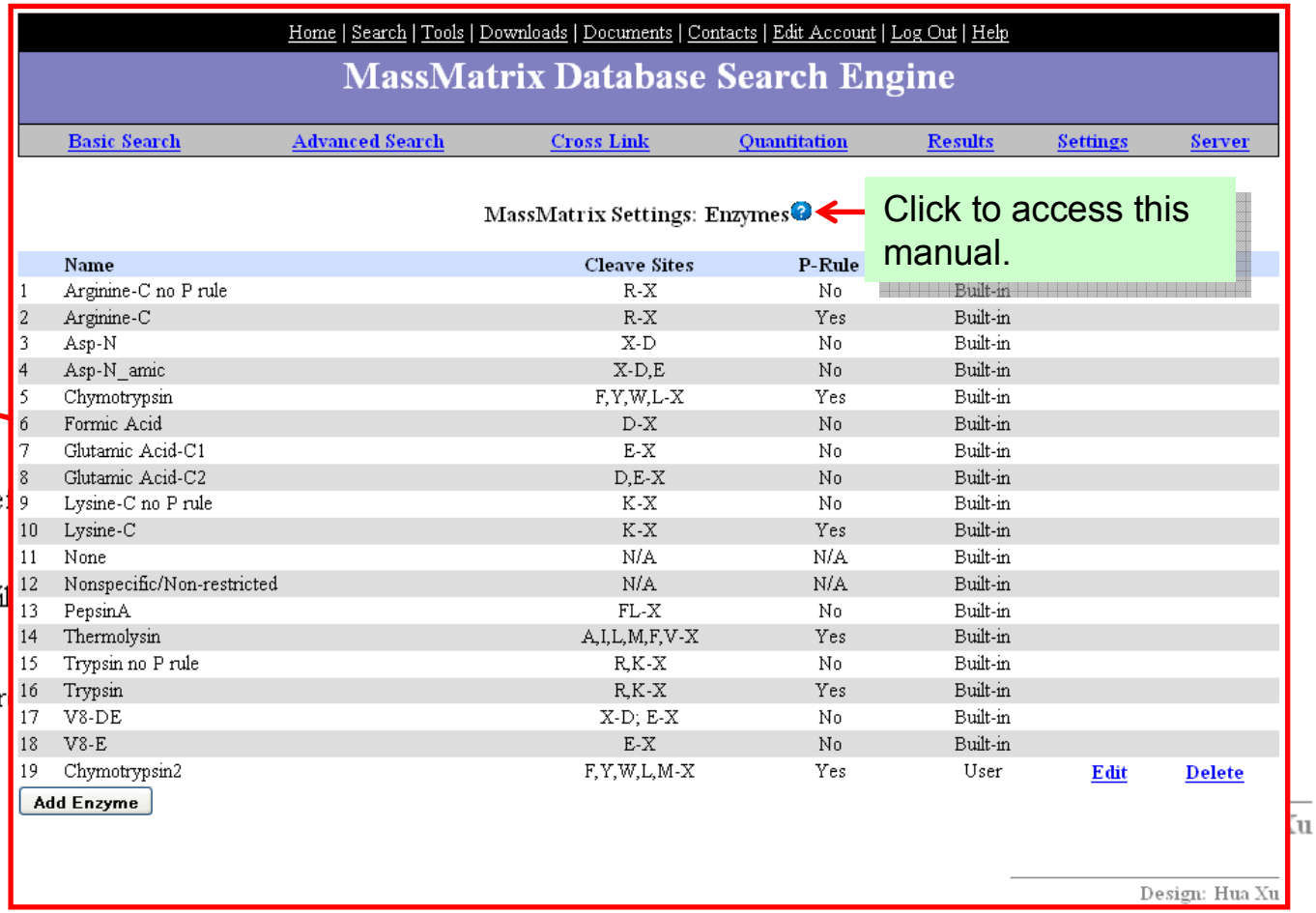
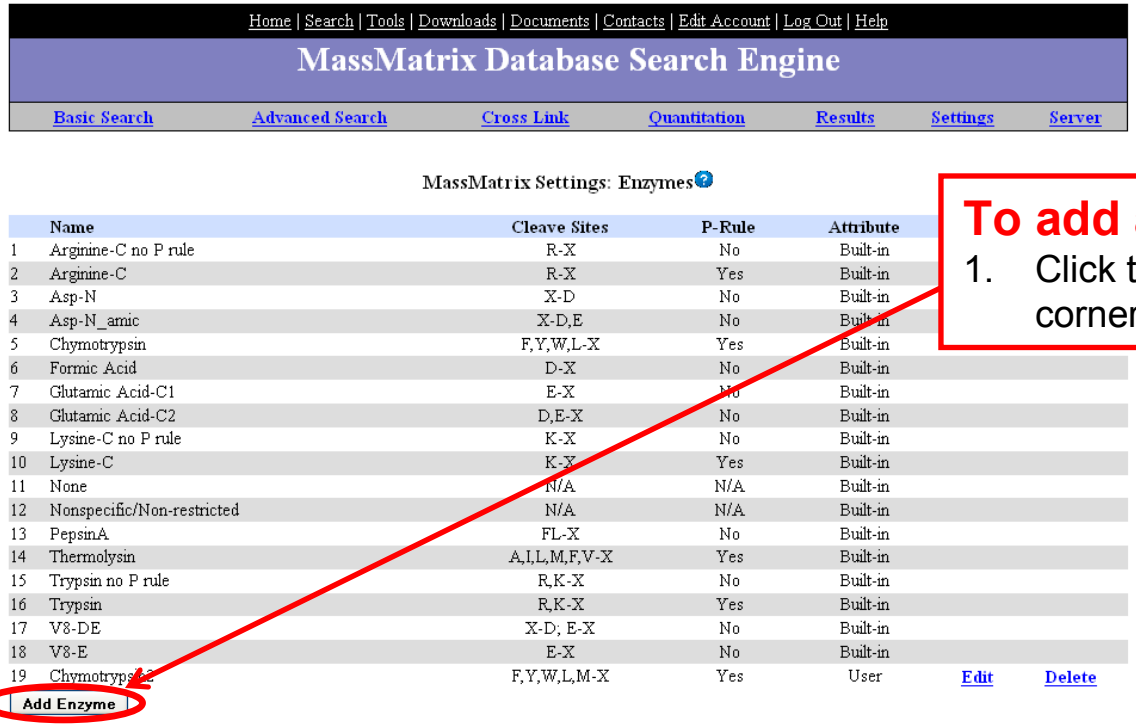
6. **SILAC**:
To add and configure SILAC quantitation pr

Click to go to enzyme configuration page



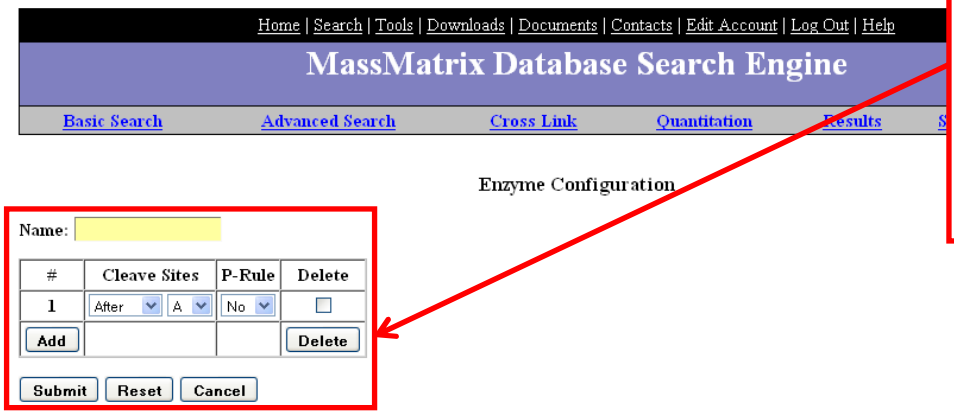
	Name	Cleave Sites	P-Rule			
1	Arginine-C no P rule	R-X	No	Built-in		
2	Arginine-C	R-X	Yes	Built-in		
3	Asp-N	X-D	No	Built-in		
4	Asp-N_amic	X-D,E	No	Built-in		
5	Chymotrypsin	F,Y,W,L-X	Yes	Built-in		
6	Formic Acid	D-X	No	Built-in		
7	Glutamic Acid-C1	E-X	No	Built-in		
8	Glutamic Acid-C2	D,E-X	No	Built-in		
9	Lysine-C no P rule	K-X	No	Built-in		
10	Lysine-C	K-X	Yes	Built-in		
11	None	N/A	N/A	Built-in		
12	Nonspecific/Non-restricted	N/A	N/A	Built-in		
13	PepsinA	FL-X	No	Built-in		
14	Thermolysin	A,I,L,M,F,V-X	Yes	Built-in		
15	Trypsin no P rule	R,K-X	No	Built-in		
16	Trypsin	R,K-X	Yes	Built-in		
17	V8-DE	X-D; E-X	No	Built-in		
18	V8-E	E-X	No	Built-in		
19	Chymotrypsin2	F,Y,W,L,M-X	Yes	User	Edit	Delete

MassMatrix Server Settings – Enzymes



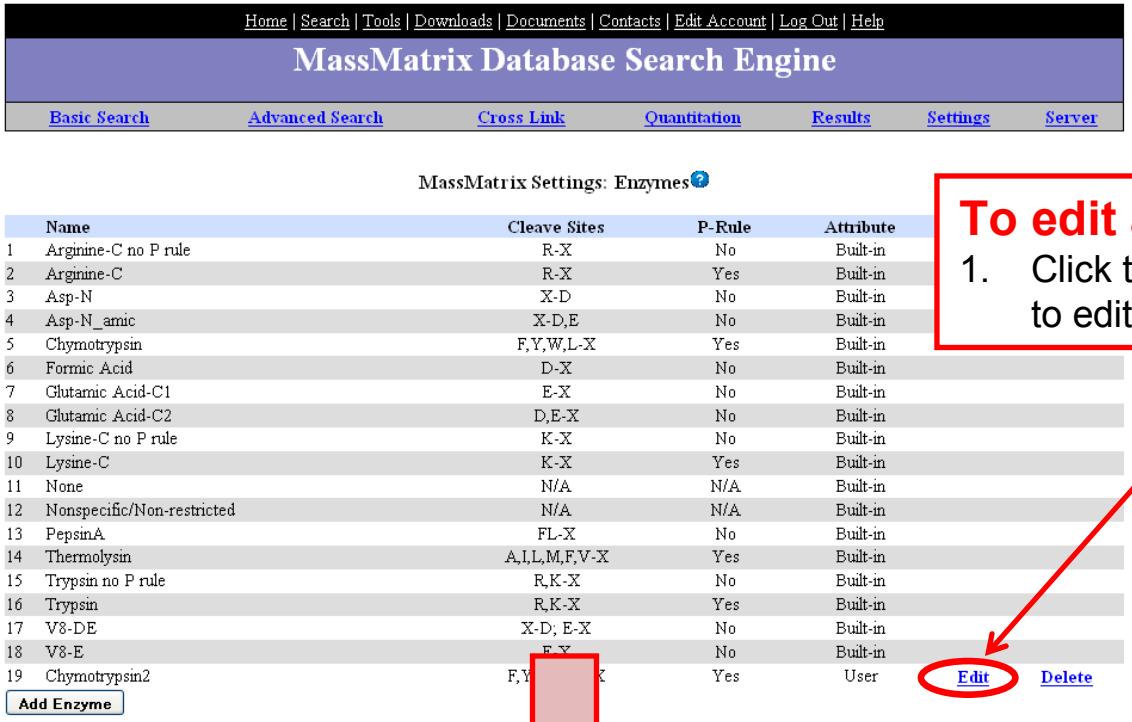
To add an enzyme:

1. Click the icon of "Add Enzyme" at the lower left corner.



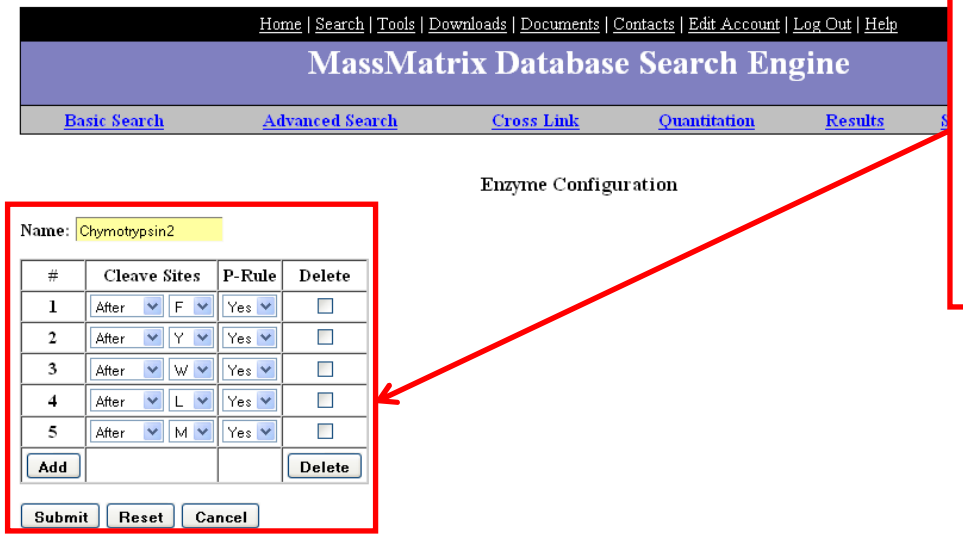
2. Specify the full name of your enzyme.
3. Specify the cleavage sites. Multiple sites can be specified. Sites can be added or deleted by the icons of "Add" and "Delete".
4. Click the icon of "Submit" to submit the changes or click the icon of "Cancel" to cancel the change.

MassMatrix Server Settings – Enzymes



To edit an enzyme:

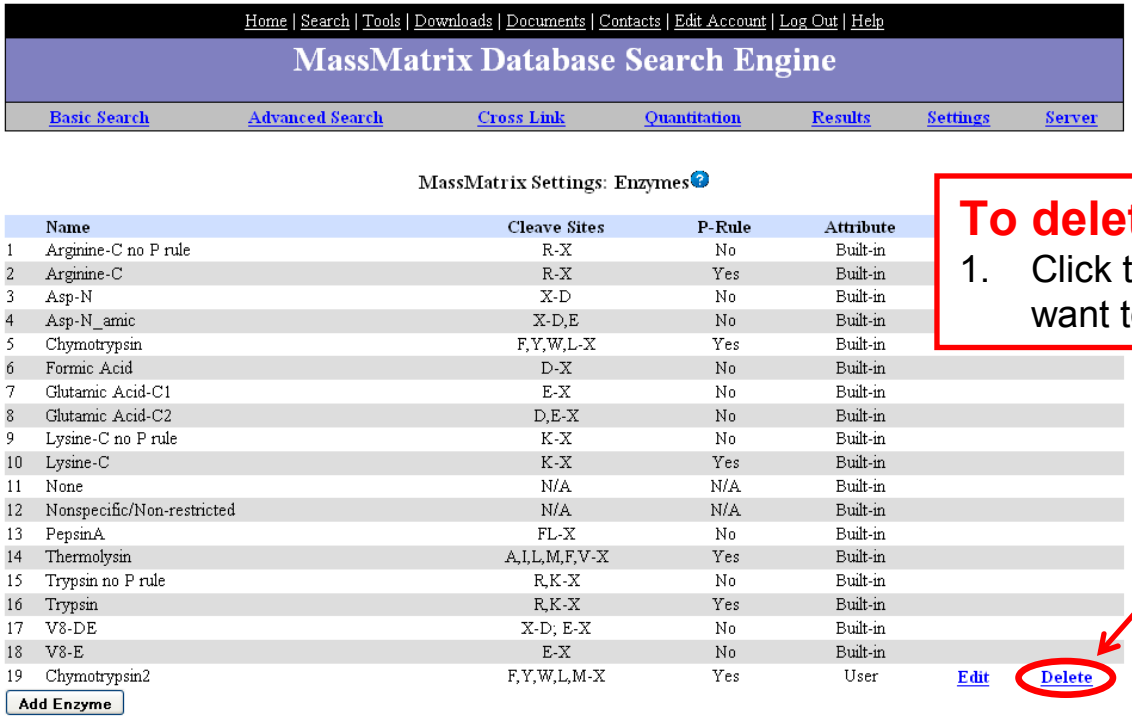
1. Click the “Edit” link of the enzyme that you want to edit.



2. Edit the full name of your enzyme.
3. Edit the cleavage sites. Multiple sites can be specified. Sites can be added or deleted by the icons of "Add" and "Delete".
4. Click the icon of "Submit" to submit the changes or click the icon of "Cancel" to cancel the change.

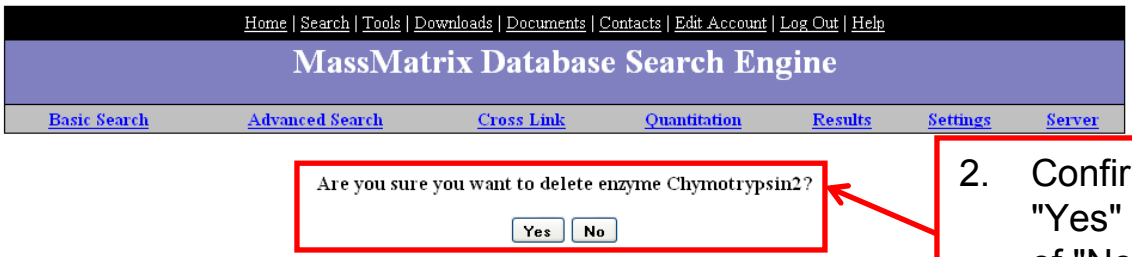
Note: Built-in enzymes cannot be edited.

MassMatrix Server Settings – Enzymes



To delete an enzyme:

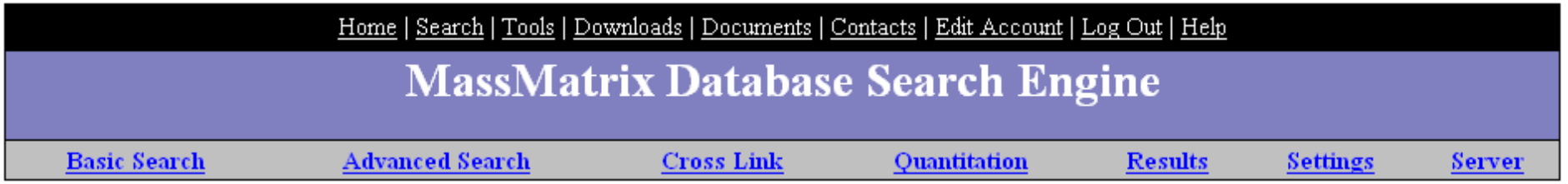
1. Click the “Delete” link of the enzyme that you want to delete.



2. Confirm the deletion by clicking the icon of "Yes" or cancel the deletion by clicking the icon of "No".

Note: Built-in enzymes cannot be deleted.

MassMatrix Server Settings – Amino Acids



1. Protein databases:
To upload custom protein databases.

2. Modifications:
To configure and add custom modifications.

3. Enzymes:
To configure and add custom enzymes.

4. Amino Acids:
To configure amino acids.
"J", "O", and "U" are available for user to def

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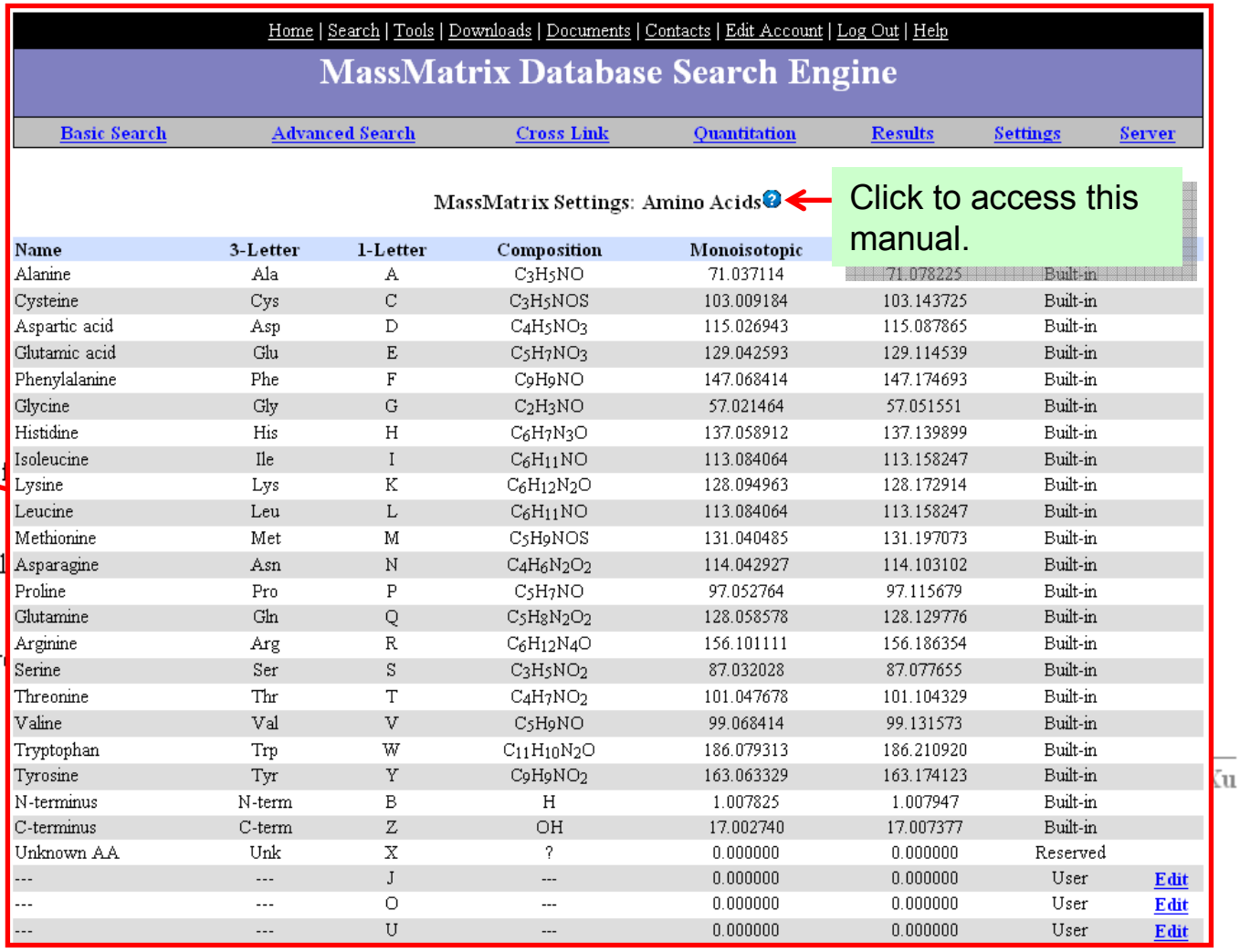
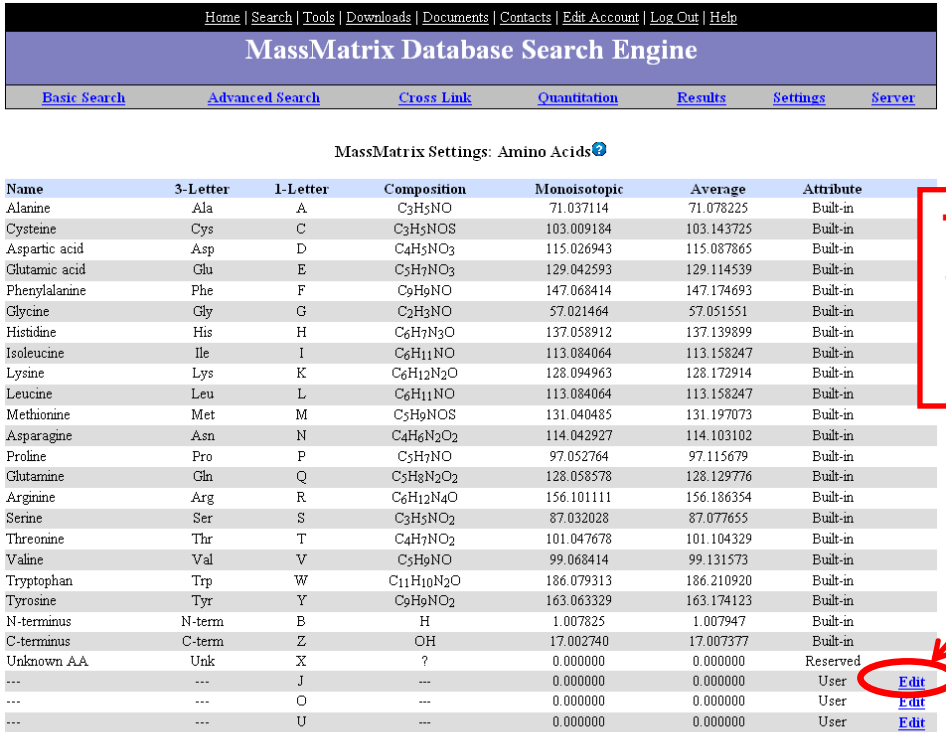
Click to go to amino acid configuration page

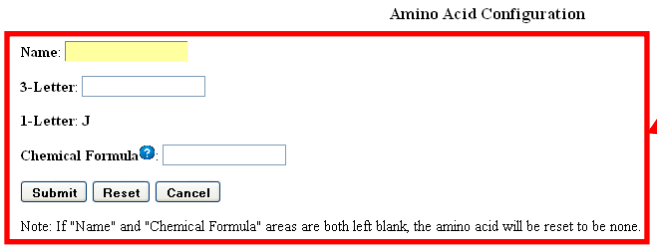


Name	3-Letter	1-Letter	Composition	Monoisotopic	Average		
Alanine	Ala	A	C_3H_5NO	71.037114	71.078225	Built-in	
Cysteine	Cys	C	C_3H_5NOS	103.009184	103.143725	Built-in	
Aspartic acid	Asp	D	$C_4H_5NO_3$	115.026943	115.087865	Built-in	
Glutamic acid	Glu	E	$C_5H_7NO_3$	129.042593	129.114539	Built-in	
Phenylalanine	Phe	F	C_9H_9NO	147.068414	147.174693	Built-in	
Glycine	Gly	G	C_2H_3NO	57.021464	57.051551	Built-in	
Histidine	His	H	$C_6H_7N_3O$	137.058912	137.139899	Built-in	
Isoleucine	Ile	I	$C_6H_{11}NO$	113.084064	113.158247	Built-in	
Lysine	Lys	K	$C_6H_{12}N_2O$	128.094963	128.172914	Built-in	
Leucine	Leu	L	$C_6H_{11}NO$	113.084064	113.158247	Built-in	
Methionine	Met	M	C_5H_9NOS	131.040485	131.197073	Built-in	
Asparagine	Asn	N	$C_4H_6N_2O_2$	114.042927	114.103102	Built-in	
Proline	Pro	P	C_5H_7NO	97.052764	97.115679	Built-in	
Glutamine	Gln	Q	$C_5H_8N_2O_2$	128.058578	128.129776	Built-in	
Arginine	Arg	R	$C_6H_{12}N_4O$	156.101111	156.186354	Built-in	
Serine	Ser	S	$C_3H_5NO_2$	87.032028	87.077655	Built-in	
Threonine	Thr	T	$C_4H_7NO_2$	101.047678	101.104329	Built-in	
Valine	Val	V	C_5H_9NO	99.068414	99.131573	Built-in	
Tryptophan	Trp	W	$C_{11}H_{10}N_2O$	186.079313	186.210920	Built-in	
Tyrosine	Tyr	Y	$C_9H_9NO_2$	163.063329	163.174123	Built-in	
N-terminus	N-term	B	H	1.007825	1.007947	Built-in	
C-terminus	C-term	Z	OH	17.002740	17.007377	Built-in	
Unknown AA	Unk	X	?	0.000000	0.000000	Reserved	
---	---	J	---	0.000000	0.000000	User	Edit
---	---	O	---	0.000000	0.000000	User	Edit
---	---	U	---	0.000000	0.000000	User	Edit

MassMatrix Server Settings – Amino Acids



Name	3-Letter	1-Letter	Composition	Monoisotopic	Average	Attribute	
Alanine	Ala	A	C_3H_5NO	71.037114	71.078225	Built-in	
Cysteine	Cys	C	C_3H_5NOS	103.009184	103.143725	Built-in	
Aspartic acid	Asp	D	$C_4H_5NO_3$	115.026943	115.087865	Built-in	
Glutamic acid	Glu	E	$C_5H_7NO_3$	129.042593	129.114539	Built-in	
Phenylalanine	Phe	F	C_9H_9NO	147.068414	147.174693	Built-in	
Glycine	Gly	G	C_2H_3NO	57.021464	57.051551	Built-in	
Histidine	His	H	$C_6H_7N_3O$	137.058912	137.139899	Built-in	
Isoleucine	Ile	I	$C_6H_{11}NO$	113.084064	113.158247	Built-in	
Lysine	Lys	K	$C_6H_{12}N_2O$	128.094963	128.172914	Built-in	
Leucine	Leu	L	$C_6H_{11}NO$	113.084064	113.158247	Built-in	
Methionine	Met	M	C_5H_9NOS	131.040485	131.197073	Built-in	
Asparagine	Asn	N	$C_4H_6N_2O_2$	114.042927	114.103102	Built-in	
Proline	Pro	P	C_5H_7NO	97.052764	97.115679	Built-in	
Glutamine	Gln	Q	$C_5H_8N_2O_2$	128.058578	128.129776	Built-in	
Arginine	Arg	R	$C_6H_{12}N_4O$	156.101111	156.186354	Built-in	
Serine	Ser	S	$C_3H_5NO_2$	87.032028	87.077655	Built-in	
Threonine	Thr	T	$C_4H_7NO_2$	101.047678	101.104329	Built-in	
Valine	Val	V	C_5H_9NO	99.068414	99.131573	Built-in	
Tryptophan	Trp	W	$C_{11}H_{10}N_2O$	186.079313	186.210920	Built-in	
Tyrosine	Tyr	Y	$C_9H_9NO_2$	163.063329	163.174123	Built-in	
N-terminus	N-term	B	H	1.007825	1.007947	Built-in	
C-terminus	C-term	Z	OH	17.002740	17.007377	Built-in	
Unknown AA	Unk	X	?	0.000000	0.000000	Reserved	
---	---	J	---	0.000000	0.000000	User	Edit
---	---	O	---	0.000000	0.000000	User	Edit
---	---	U	---	0.000000	0.000000	User	Edit



To edit an amino acid:

1. Click the "Edit" link of the amino acid that you want to edit. Note: only "J", "O" , and "U" can be edited.

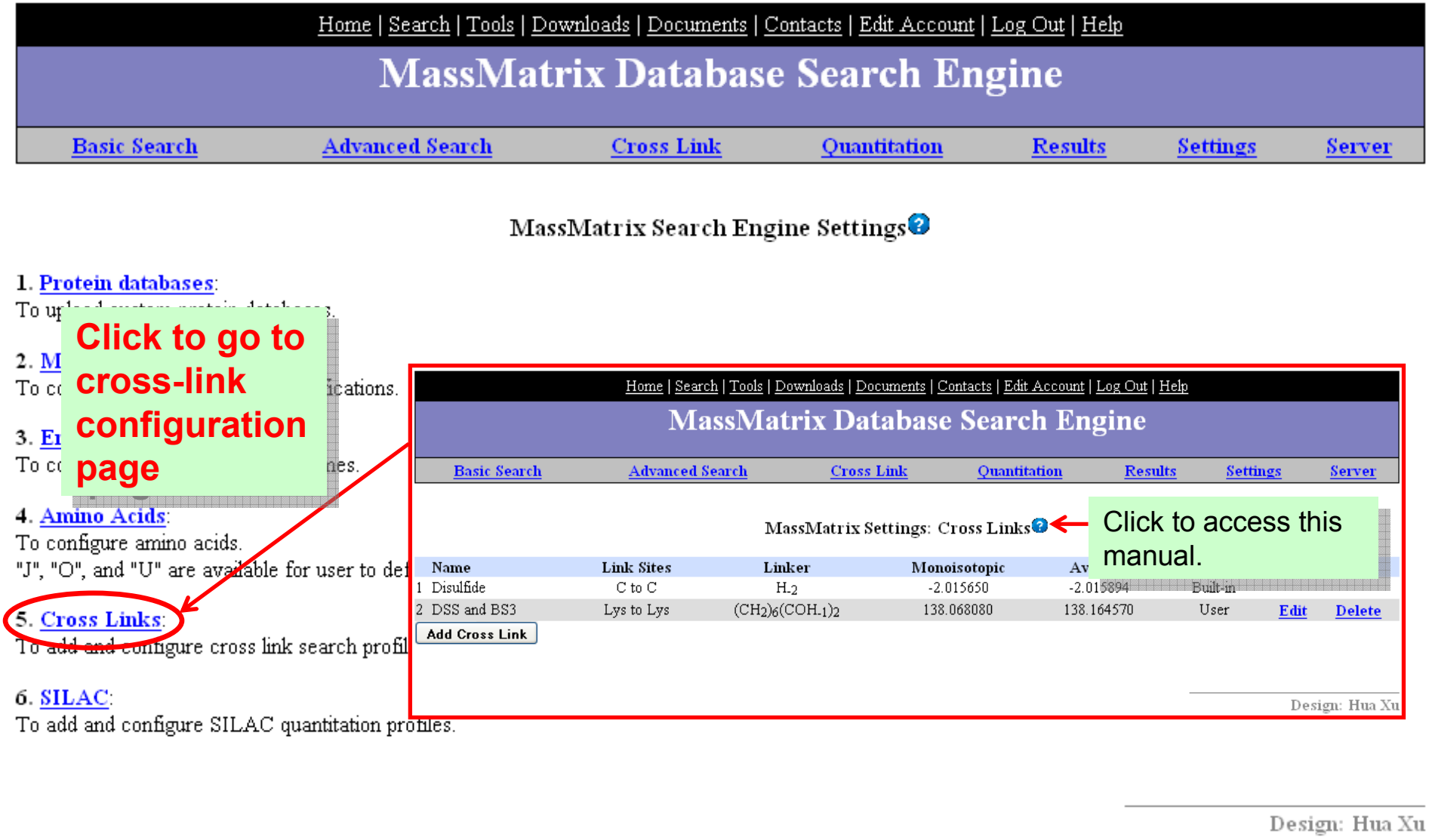
2. Edit the full name, 3-letter name, and chemical formula of the amino acid (click the link below for chemical formula syntax).

http://sourceforge.net/projects/massmatrix/files/MassMatrix_Manuals/Chemical%20Formula%20Syntax.pdf/download

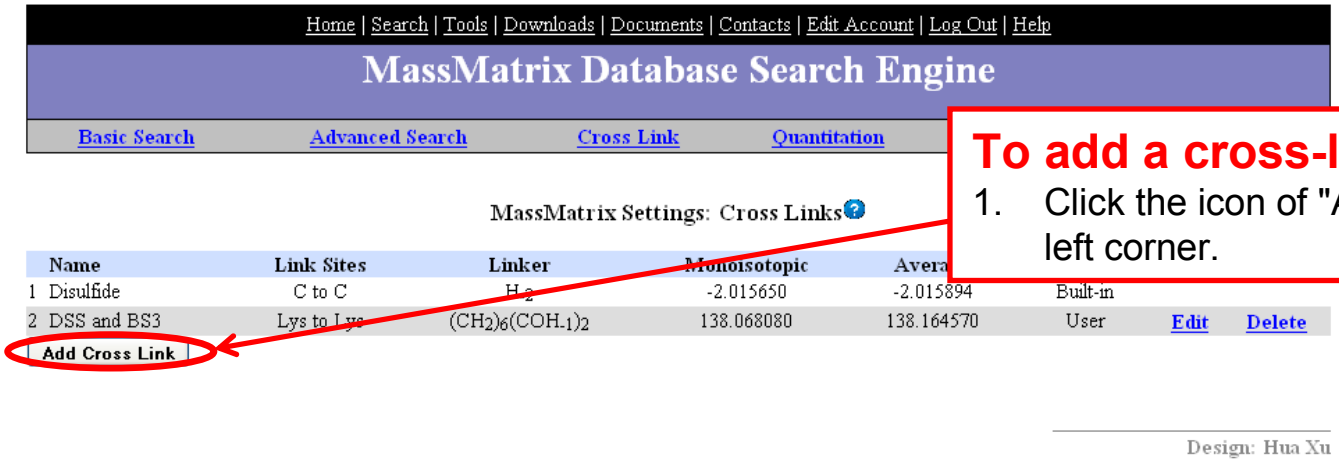
3. Click the icon of "Submit" to submit the changes or click the icon of "Cancel" to cancel the change.

Note: Amino acids cannot be added or deleted.

MassMatrix Server Settings – Cross Links

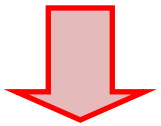


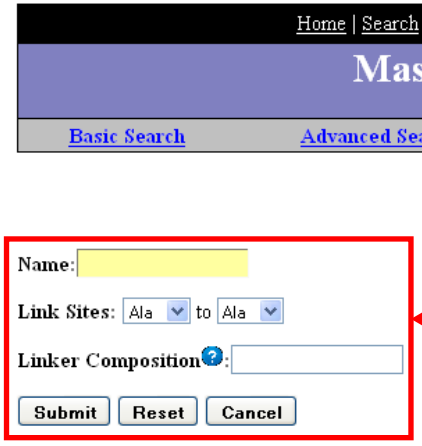
MassMatrix Server Settings – Cross Links



To add a cross-link:

1. Click the icon of "Add Cross Link" at the lower left corner.





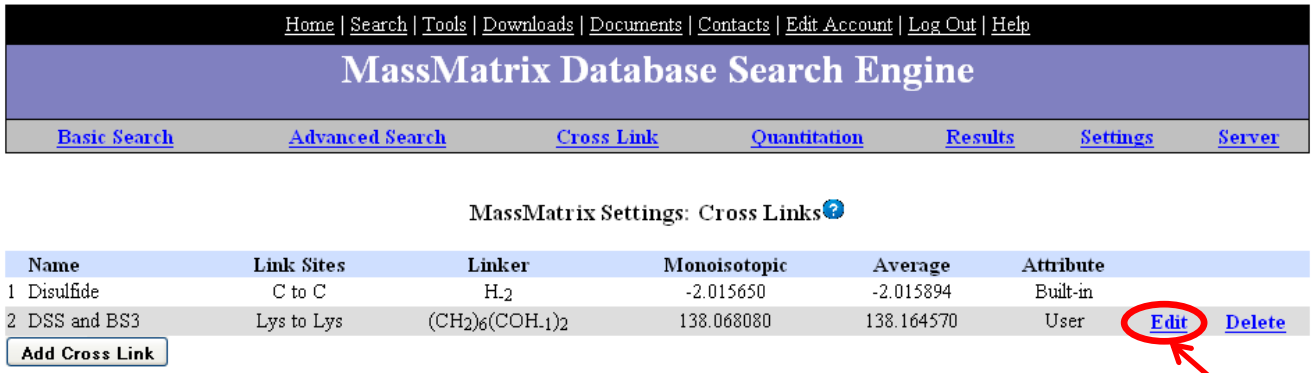
2. Specify the name of the cross link.
3. Select the two link site residues.
4. Specify the chemical formula of the cross linker (click the link below for chemical formula syntax).

http://sourceforge.net/projects/massmatrix/files/MassMatrix_Manuals/Chemical%20Formula%20Syntax.pdf/download

This will be used to calculate the mass shift to the peptides caused by the cross link.

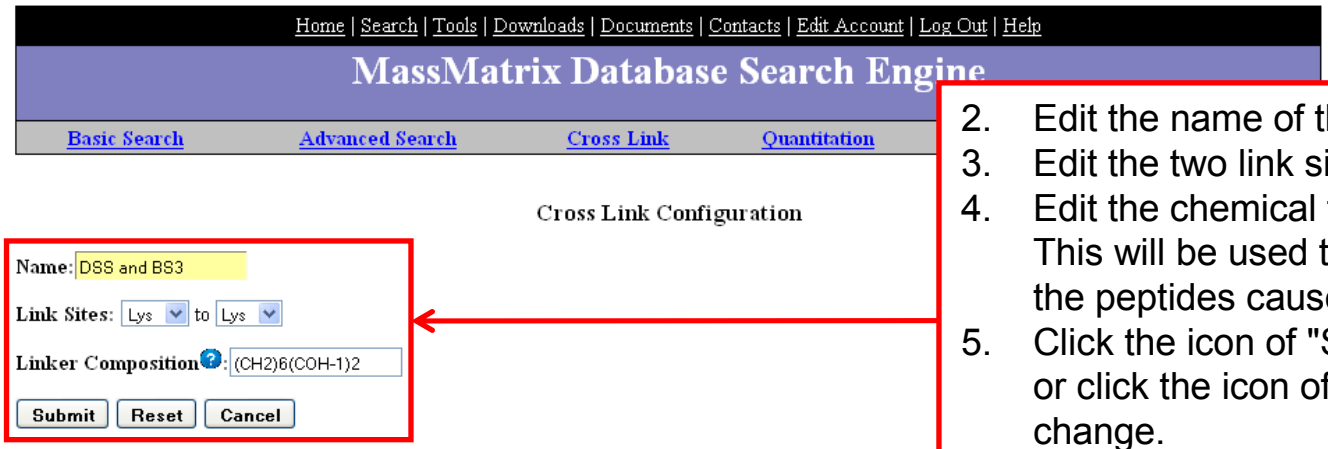
5. Click the icon of "Submit" to submit the changes or click the icon of "Cancel" to cancel the change.

MassMatrix Server Settings – Cross Links



To edit a cross-link:

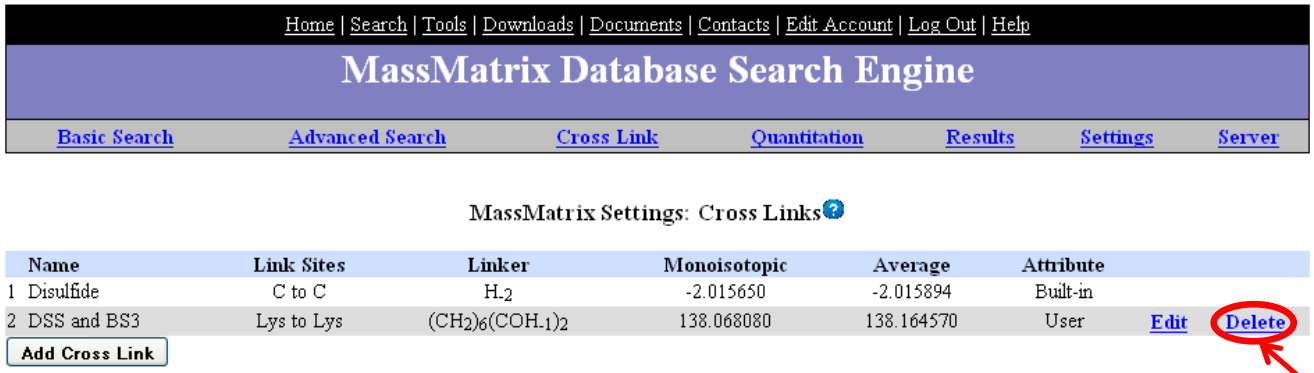
1. Click the "Edit" link of the cross-link that you want to edit.



2. Edit the name of the cross link.
3. Edit the two link site residues.
4. Edit the chemical formula of the cross linker. This will be used to calculate the mass shift to the peptides caused by the cross link.
5. Click the icon of "Submit" to submit the changes or click the icon of "Cancel" to cancel the change.

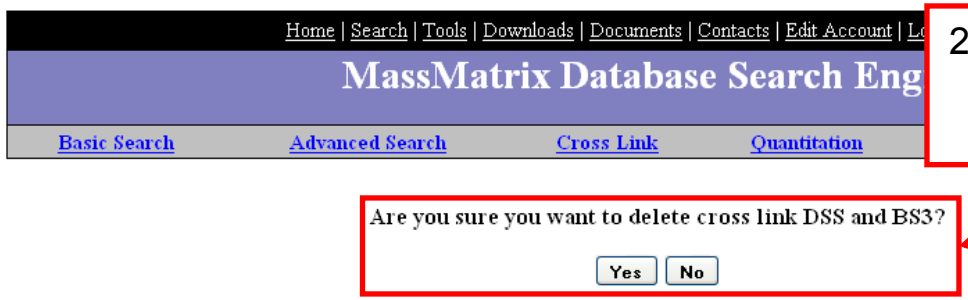
Note: Disulfide bond is built-in and cannot be edited.

MassMatrix Server Settings – Cross Links



To delete a cross-link:

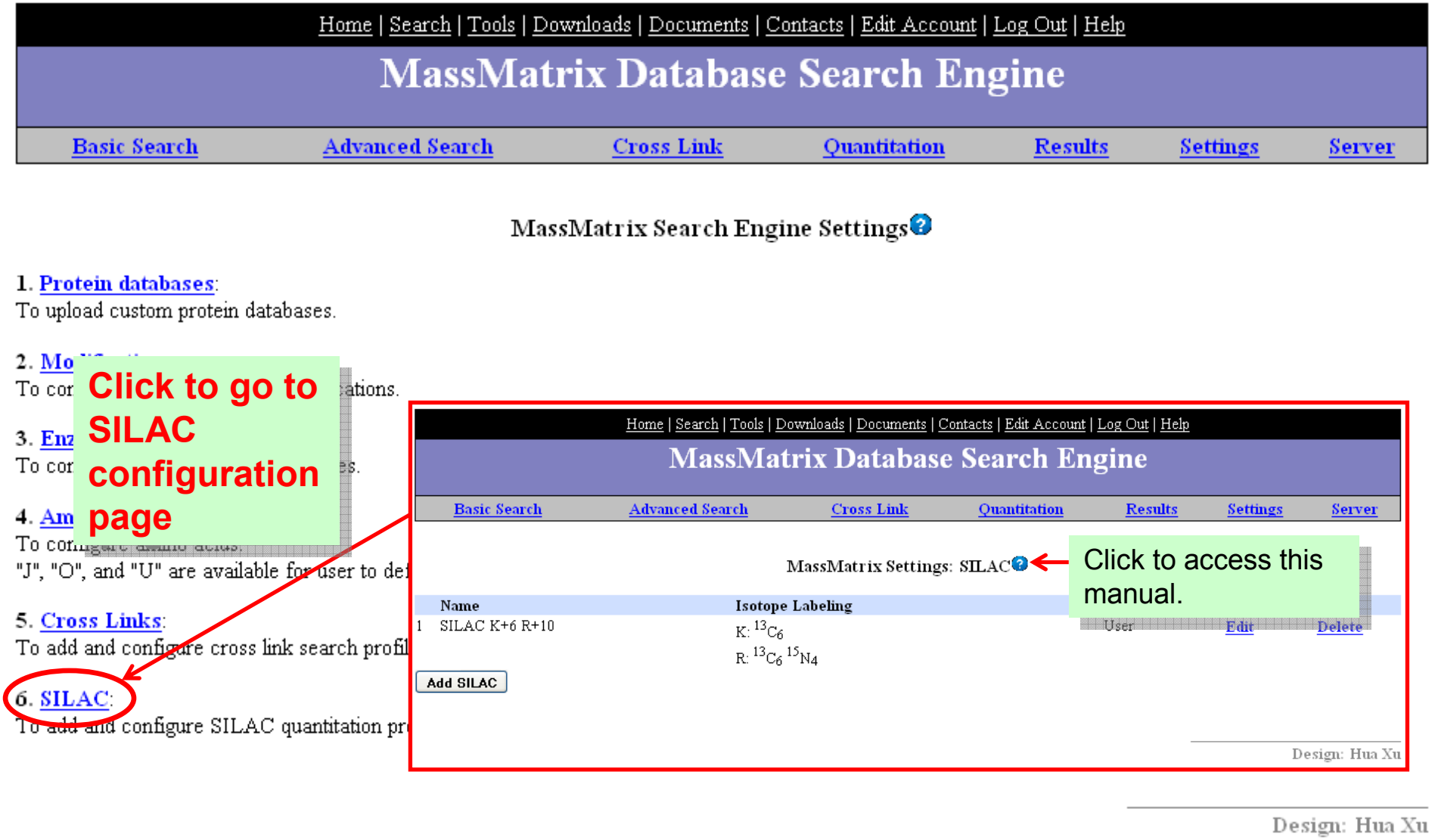
1. Click the “Delete” link of the cross-link that you want to delete.



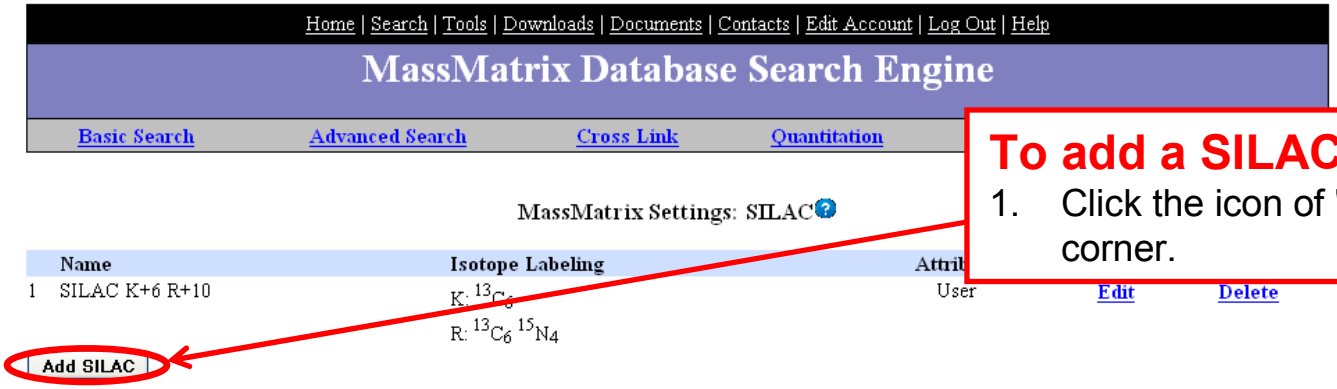
2. Confirm the deletion by clicking the icon of "Yes" or cancel the deletion by clicking the icon of "No".

Note: Disulfide bond is built-in and cannot be deleted.

MassMatrix Server Settings – SILAC

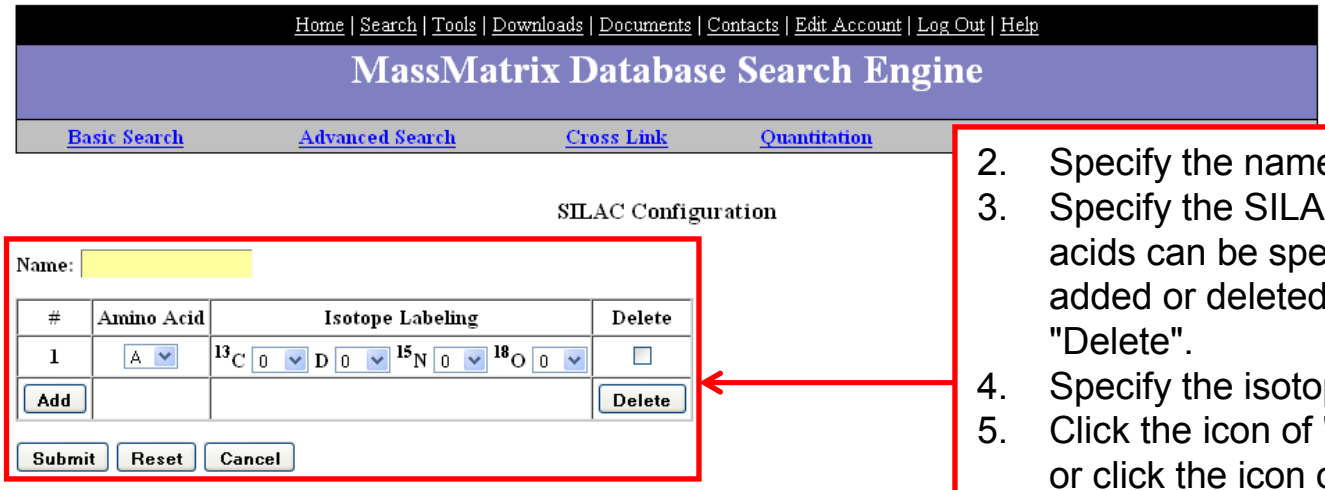


MassMatrix Server Settings – SILAC



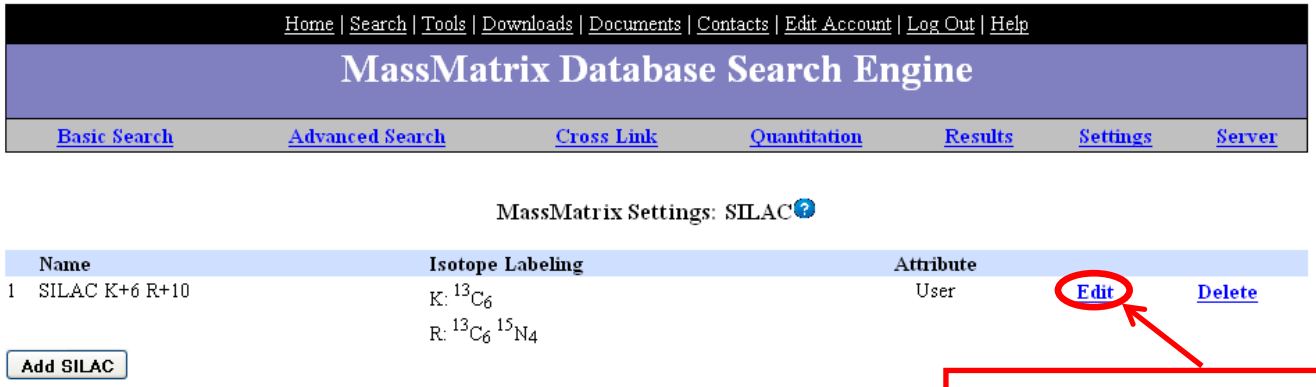
To add a SILAC method:

1. Click the icon of "Add SILAC" at the lower left corner.



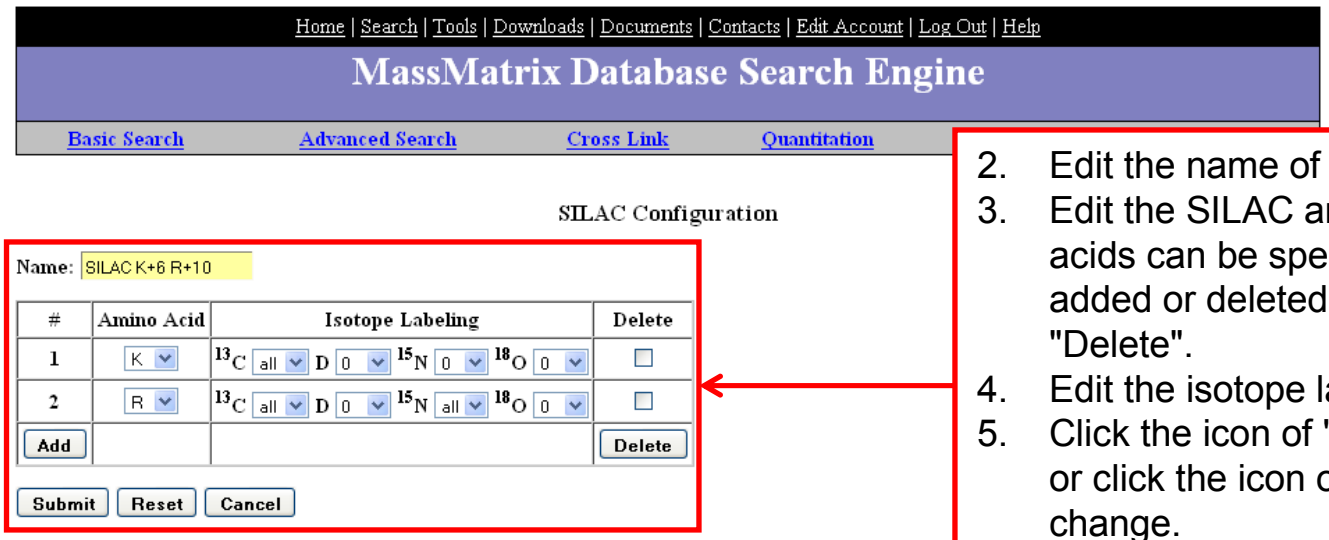
2. Specify the name of the SILAC.
3. Specify the SILAC amino acids. Multiple amino acids can be specified. Amino acids can be added or deleted by the icons of "Add" and "Delete".
4. Specify the isotope labeling for the amino acid.
5. Click the icon of "Submit" to submit the changes or click the icon of "Cancel" to cancel the change.

MassMatrix Server Settings – SILAC



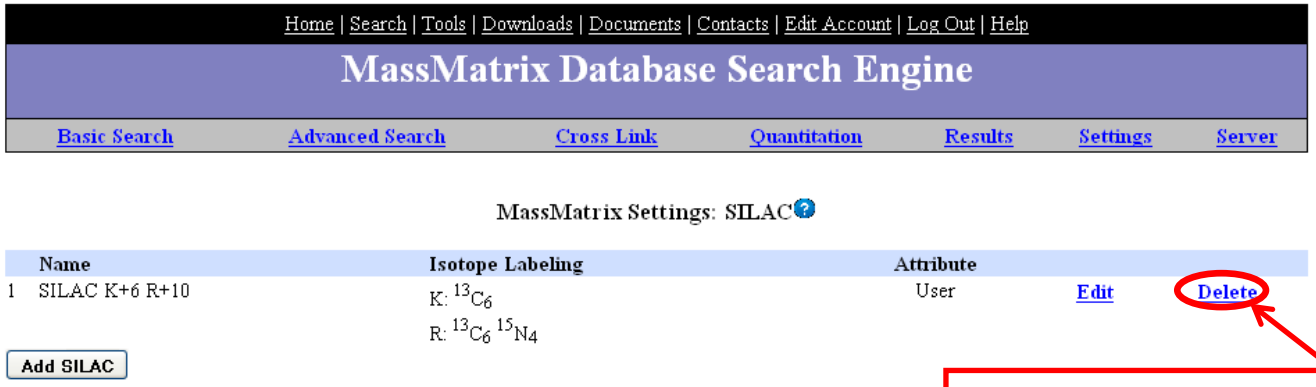
To edit a SILAC method:

1. Click the "Edit" link of the SILAC method that you want to edit.



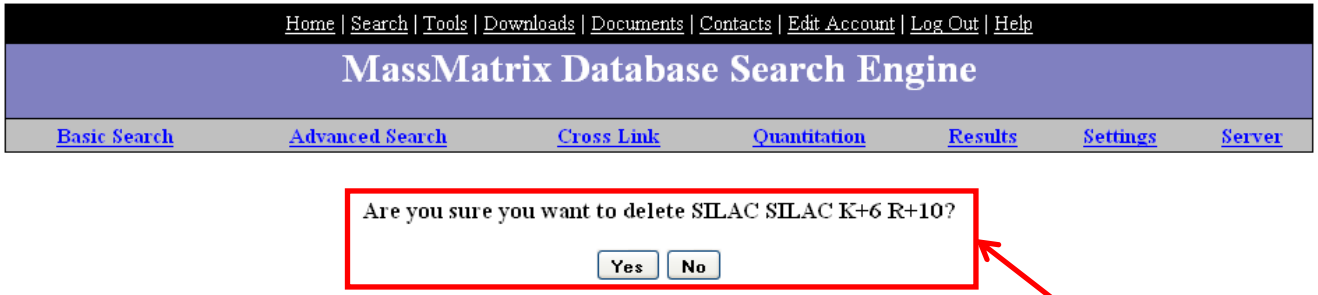
2. Edit the name of the SILAC.
3. Edit the SILAC amino acids. Multiple amino acids can be specified. Amino acids can be added or deleted by the icons of "Add" and "Delete".
4. Edit the isotope labeling for the amino acid.
5. Click the icon of "Submit" to submit the changes or click the icon of "Cancel" to cancel the change.

MassMatrix Server Settings – SILAC



To delete a SILAC method:

1. Click the “Delete” link of the SILAC method that you want to delete.

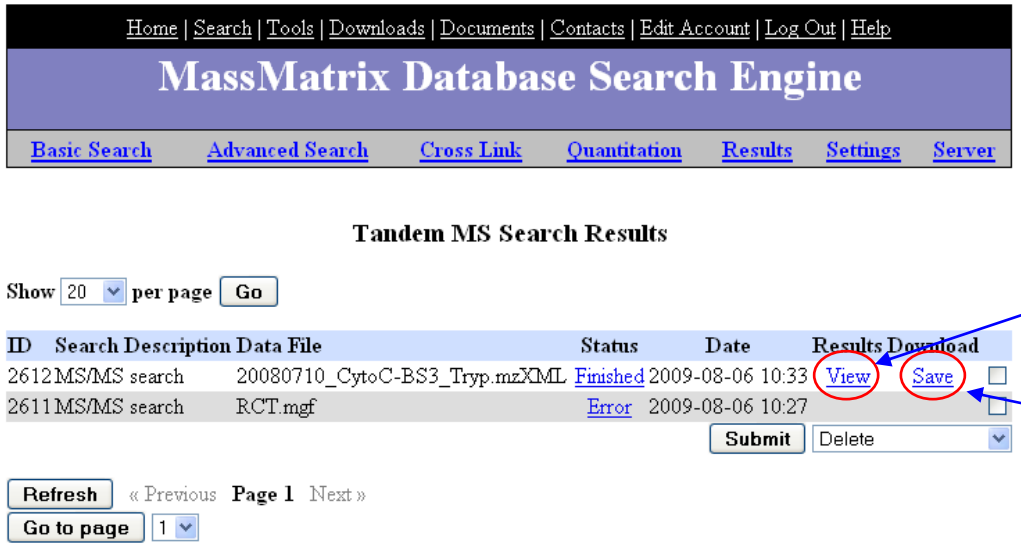


2. Confirm the deletion by clicking the icon of "Yes" or cancel the deletion by clicking the icon of "No".

6. MassMatrix Search Results

Open MassMatrix Search Results

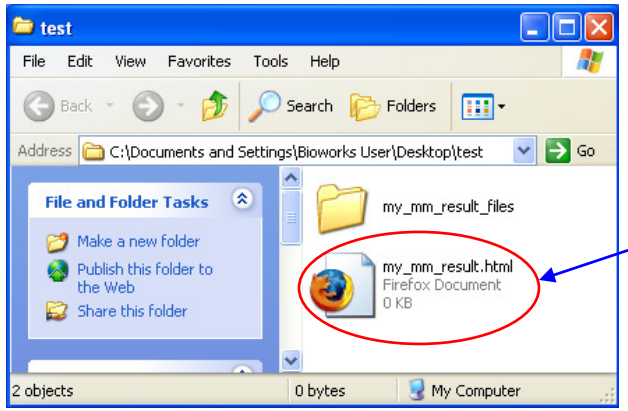
1. View MassMatrix search results online



Click to view your results online

Click to save your results

2. Open MassMatrix search results locally



Double click to open your results locally

Main Html

MassMatrix Searching Results

Input Parameters

version	: MassMatrix 2.2.2, Jul 23 2009
Tandem MS/MS data file	: 112806_BOVINE_std.mgf
Database	: bovine_histones.fasta
Decoy sequences	: reversed
Digestion	: Trypsin
fragmentation	: CID
Non-monoisotopic ions	: no
Modifications	: none
Fixed Modifications	: none
Maximum # Missed Cleavages	: 2
Maximum Length of Peptides	: 40
Minimum Length of Peptides	: 6
Peptide Mass Tolerance	: ±2.00 Da
Fragment Mass Tolerance	: ±0.80 Da
Mass	: monoisotopic
Minimum Score of Output	: 10
Minimum pp value of Output	: 5.0
Minimum pp2value of Output	: 5.0
Minimum PP_{tag} of output	: 1.3
Minimum CLpp of Output	: 0.0
Minimum CLpp2 of Output	: 0.0
Minimum protein score	: 5.0
Max # PTM per peptide	: 2
Maximum # of matches/Spec	: 1
Maximum # of combs/peptide	: 1
Cross linkage search	: Disabled
Total # of MS/MS spectra	: 3152
Protein sequences checked	: 234
Peptide sequences checked	: 6717
Peptides checked	: 6.717000e+03
R^2 of LR model for t_R vs H	: N/A or failed
MS/MS tag quantitation	: disabled
Wall clock time	: 0hr 0min 9sec
Date and time	: Thu Jul 23 21:49:24 2009

Input Parameters. This section contains all the parameters used during the search. Searching parameters may affect your search results dramatically. So it is very important that you keep all the parameters that you use during the searches and some proteomics journals may require you to report those parameters including the version of MassMatrix that you are using.

Note. This section contains a very brief note about how to understand and interpret peptide and protein scores in MassMatrix. The definition of those scores are available in the following publications:

1) Hua Xu, Michael A. Freitas BMC Bioinformatics 2007, 8, 133

2) Hua Xu, Michael A. Freitas J. Proteome Res. 2008, 7(7), 2605-2615

User commments

MS/MS search

User Comments.

Note

The quality of a peptide match is mainly evalulated by three statistical scores: **pp**, **pp_2**, **pp_{tag}**.

Based on the search space calculated by MassMatrix for the protein database and search mass tolerances,

$10^{-\{\max(pp,pp_2)-1.1\}}$ *gives the probability that a peptide match is a random occurance;*

$10^{-pp_{tag}}$ *gives the probability that a peptide match has a random pattern of AA residue tags;*

A peptide match with $\max(pp,pp_2) > 2.4$ and $pp_{tag} > 1.3$ is considered to be significant with p value < 0.05.

$\max(pp,pp_2)$ *is the maximum value of* **pp** *and* **pp_2** *values.*

The quality of a protein hit is mainly evaluated by **protein score**. *If the decoy database is included dring the search, false discovery rate will be evaluated by* **decoy%**. *An* **decoy%** *< 2.5% indicates the protein hit is significant.*

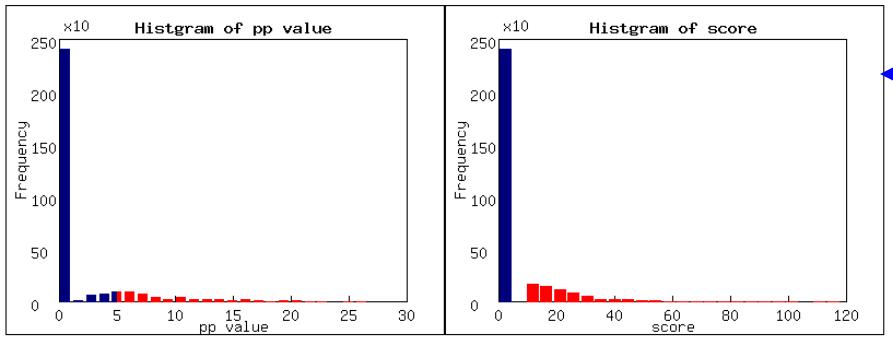
Citation

If you include MassMatrix search results in a publication, please cite

1. Hua Xu, Michael A. Freitas *BMC Bioinformatics* **2007**, 8, 133 Link
2. Hua Xu, Michael A. Freitas *J. Proteome Res.* **2008**, 7, 138-144 Link
3. Hua Xu, Liwen Zhang, Michael A. Freitas *J. Proteome Res.* **2008**, 7, 2605-2615 Link
4. Hua Xu, Lanhao Yang, Michael A. Freitas *BMC Bioinformatics* **2008**, 9, 347 Link

Citation. This section contains the publications of MassMatrix that you need to cite if you are using results from MassMatrix in your publications.

Main Html – Con't



Histograms of pp values and scores for all peptide matches in the search.

Protein Hit List. This section contains the list of protein matches with significant scores for the search.

Protein Hit List

hit#	score	decoy%	protein description
hit1	357	0.00%	IPI:IPI00689632.5|SWISS-PROT:Q32L48|REFSEQ:NP_001075211 Tax_Id=9913 Gene_Symbol=LOC614958 Histone H2B type 1-
hit2	329	0.00%	IPI:IPI00730950.1|REFSEQ:XP_873227 Tax_Id=9913 Gene_Symbol=LOC616167 similar to histone H4
hit3	307	0.00%	IPI:IPI00690163.1|REFSEQ:XP_871891 Tax_Id=9913 Gene_Symbol=LOC615091 similar to histone H2b-616
hit4	307	0.00%	IPI:IPI00713959.6|SWISS-PROT:Q2M2T1|REFSEQ:NP_001071531 Tax_Id=9913 Gene_Symbol=HIST1H2BK Histone H2B type 1-
hit5	292	0.00%	IPI:IPI00694637.2|REFSEQ:XP_610495 Tax_Id=9913 Gene_Symbol=LOC531990 similar to histone protein Hist2h3c1
hit6	259	0.00%	IPI:IPI00705000.1|REFSEQ:XP_601249 Tax_Id=9913 Gene_Symbol=LOC522960 similar to histone 1, H2bk
hit7	251	0.00%	IPI:IPI00714808.2|REFSEQ:XP_583411 Tax_Id=9913 Gene_Symbol=LOC506900 similar to histone H2A isoform 1
hit8	233	0.00%	IPI:IPI00708769.1|TREMBL:Q3ZBX9|ENSEMBL:ENSBTAP00000040641|REFSEQ:NP_001071557;XP_001249755 Tax_Id=9913 Gene_
hit9	229	0.00%	IPI:IPI00728286.2|ENSEMBL:ENSBTAP00000043359|REFSEQ:XP_871754 Tax_Id=9913 Gene_Symbol=LOC614991 similar to hi
hit10	222	0.00%	IPI:IPI00716205.3|ENSEMBL:ENSBTAP00000028147|REFSEQ:XP_604589 Tax_Id=9913 Gene_Symbol=LOC526226 similar to hi
hit11	221	0.00%	IPI:IPI00733634.1|REFSEQ:XP_870427 Tax_Id=9913 Gene_Symbol=LOC614089 similar to histone H2B
hit12	190	0.00%	IPI:IPI00698750.2|ENSEMBL:ENSBTAP00000005884|REFSEQ:XP_001250110 Tax_Id=9913 Gene_Symbol=LOC781916 similar tc
hit13	179	0.00%	IPI:IPI00827006.2|ENSEMBL:ENSBTAP00000015499|REFSEQ:XP_586208 Tax_Id=9913 Gene_Symbol=LOC509275 similar to Hi
hit14	167	0.00%	IPI:IPI00698958.3|REFSEQ:XP_871733 Tax_Id=9913 Gene_Symbol=LOC614974 similar to histone H2A
hit15	153	0.00%	IPI:IPI00702167.2|REFSEQ:XP_875272 Tax_Id=9913 Gene_Symbol=L
hit16	137	0.00%	IPI:IPI00732556.2|ENSEMBL:ENSBTAP00000002124;ENSBTAP00000042
hit17	133	0.00%	IPI:IPI00692540.3|ENSEMBL:ENSBTAP00000005876|REFSEQ:XP_87159
hit18	126	0.00%	IPI:IPI00839837.1|ENSEMBL:ENSBTAP00000040992|REFSEQ:XP_87488
hit19	123	0.00%	IPI:IPI00703727.1|ENSEMBL:ENSBTAP00000019531|REFSEQ:XP_60569
hit20	118	0.00%	IPI:IPI00711951.4|TREMBL:Q9TRT8|ENSEMBL:ENSBTAP00000036903 T
hit21	113	0.00%	IPI:IPI00690979.3|REFSEQ:XP_869143 Tax_Id=9913 Gene_Symbol=L
hit22	108	0.00%	IPI:IPI00693931.2|SWISS-PROT:P0C0S4|REFSEQ:NP_777234 Tax_Id=
hit23	108	0.00%	IPI:IPI00699981.3|REFSEQ:XP_875151 Tax_Id=9913 Gene_Symbol=LOC617745 similar to histone H2B
hit24	64	0.00%	IPI:IPI00695872.4|ENSEMBL:ENSBTAP00000040391|REFSEQ:XP_585205 Tax_Id=9913 Gene_Symbol=LOC539148 similar to hi
hit25	64	0.00%	IPI:IPI00723001.2|REFSEQ:XP_873594 Tax_Id=9913 Gene_Symbol=LOC616469 similar to histone H2B
hit26	56	0.00%	IPI:IPI00721527.2|ENSEMBL:ENSBTAP00000046556|REFSEQ:XP_001255573;XP_608871 Tax_Id=9913 Gene_Symbol=LOC788538;
hit27	46	0.00%	IPI:IPI00689641.3|REFSEQ:XP_589328 Tax_Id=9913 Gene_Symbol=LOC511901 similar to histone H1x
hit28	37	0.00%	IPI:IPI00717335.2|REFSEQ:XP_869927 Tax_Id=9913 Gene_Symbol=LOC613752 similar to histone H2B
hit29	37	0.00%	IPI:IPI00710928.2|REFSEQ:XP_001253913 Tax_Id=9913 Gene_Symbol
hit30	24	--	##DECOY## IPI:IPI00705035.2|REFSEQ:XP_590158 Tax_Id=9913 Gen
hit31	24	--	##DECOY## IPI:IPI00701086.3|TREMBL:Q2KII5|ENSEMBL:ENSBTAP000
hit32	24	--	##DECOY## IPI:IPI00699672.3|REFSEQ:XP_870957 Tax_Id=9913 Gen

Minority Report. The link to the minor report for protein matches, a list of protein matches of scores smaller than a critical value.

The link to **Peptide Match Summary**, a list of all peptide matches grouped by their sequences.

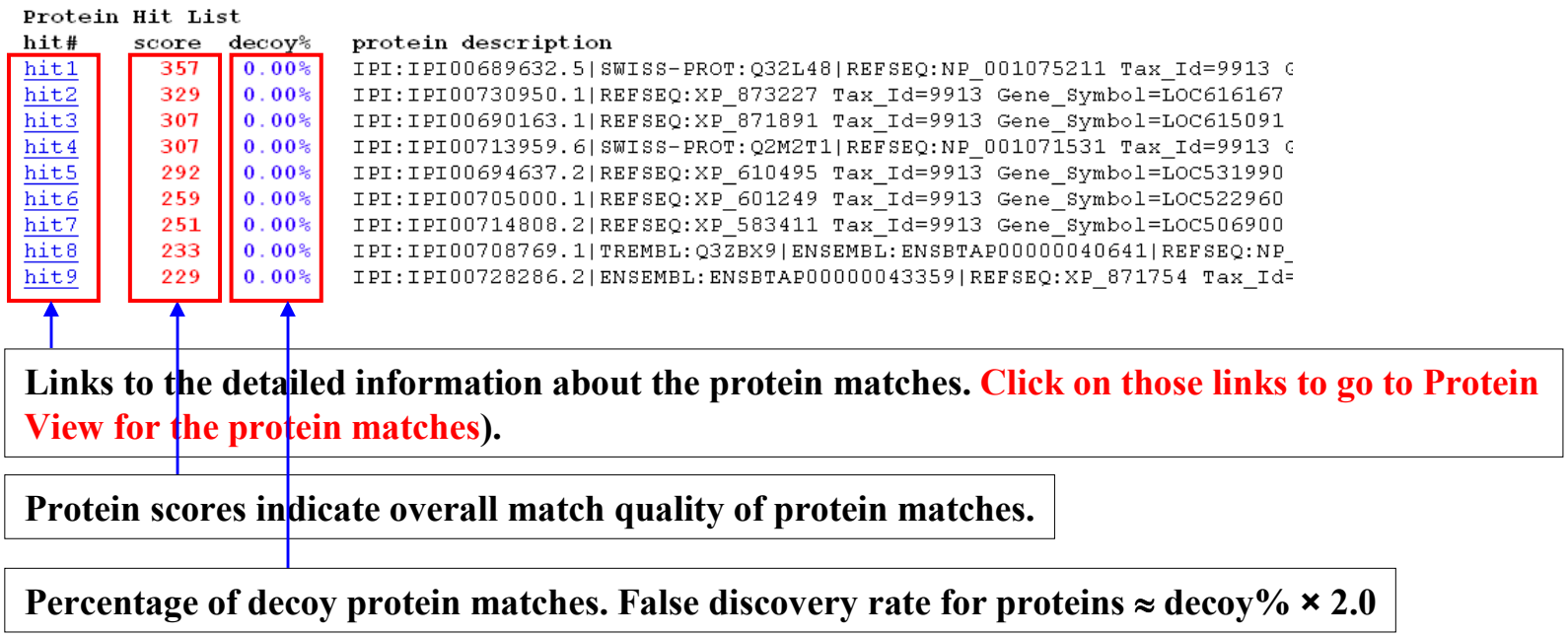
Minority Report (Minor protein hits with scores less than the automatic threshold of 21)

Peptide Match Summary (A complete list of peptide matches grouped by sequences)

Spec Summary (A complete list of peptide matches grouped by spectra)

The link to **Spec Summary**, a list of all peptide matches grouped by their experimental spectra.

Main Html – Protein List



Protein Hit List

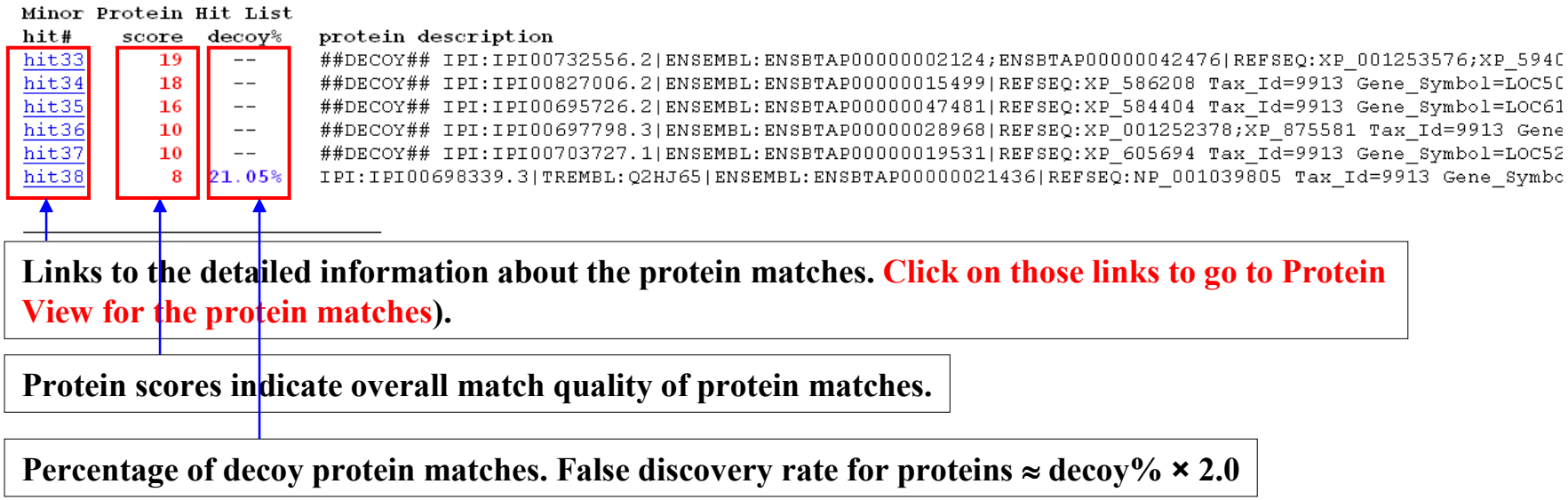
hit#	score	decoy%	protein description
hit1	357	0.00%	IPI:IPI00689632.5|SWISS-PROT:Q32L48|REFSEQ:NP_001075211 Tax_Id=9913 G
hit2	329	0.00%	IPI:IPI00730950.1|REFSEQ:XP_873227 Tax_Id=9913 Gene_Symbol=LOC616167
hit3	307	0.00%	IPI:IPI00690163.1|REFSEQ:XP_871891 Tax_Id=9913 Gene_Symbol=LOC615091
hit4	307	0.00%	IPI:IPI00713959.6|SWISS-PROT:Q2M2T1|REFSEQ:NP_001071531 Tax_Id=9913 G
hit5	292	0.00%	IPI:IPI00694637.2|REFSEQ:XP_610495 Tax_Id=9913 Gene_Symbol=LOC531990
hit6	259	0.00%	IPI:IPI00705000.1|REFSEQ:XP_601249 Tax_Id=9913 Gene_Symbol=LOC522960
hit7	251	0.00%	IPI:IPI00714808.2|REFSEQ:XP_583411 Tax_Id=9913 Gene_Symbol=LOC506900
hit8	233	0.00%	IPI:IPI00708769.1|TREMBL:Q3ZBX9|ENSEMBL:ENSBTAP00000040641|REFSEQ:NP_
hit9	229	0.00%	IPI:IPI00728286.2|ENSEMBL:ENSBTAP00000043359|REFSEQ:XP_871754 Tax_Id=

The protein hit list in the main html page contains the list of protein matches that have significant protein scores. The score, decoy rate and description of each protein match (or hit) are listed for your reference. The score evaluates the overall quality of a protein match. The list is sorted according to the protein scores. The decoy rate of a protein match is estimated from the target-decoy search strategy and can be converted to false discovery rate for that protein match using the following equation

False Discovery Rate ≈ Decoy Rate × 2.

Decoy rates are not available for decoy protein matches due to the reason that decoy protein matches are always false positives. A decoy rate of 2.5%, i.e. a false discovery rate of 5.0%, for a protein match means that the protein match is considered to be a good match at a confidence level of 95%.

Minority Report – Protein Matches with Low Scores



The minor report of a search is a list of protein matches of scores smaller than a critical value. The critical value is automatically determined by the program based on the sizes of data set and protein database. This report gives the user a chance to access those low quality protein matches that might have valuable information.

Protein View – Details of A Protein Match

HIT 1

Protein Mass: 11694.136 (monoisotopic) 11701.468(average) Protein Score: 2153 Protein pp: 10729.6
CYC_HORSE Cytochrome c

Sequence:
001 GDVEKGKKIF VQKCAQCHTV EKGGKHKTGP NLHGLFGRKT GQAPGFTYTD ANKNKGITWK EETLMEYLEN PKKYIPGTKM IFAGIKKKTE REDLIAYLKK 100
101 ATNE 104

Sequence Coverage: 86%
Sequence Tag Coverage: 84%

Index	scan#	charge	score	pp	pp2	ppTag	m/z	MW(obs)	MW	delta	miss	Unique	sequence + modifications [start:end]
188	2090	+2	58	5.7	16.3	3.3	543.3395	1085.6718	1085.6717	0.0001	2	√	GK($1)K($1)IFVQK [6:13]
636	973	+2	30	8.2	12.8	5.9	587.8836	1174.7599	1174.7558	0.0041	2	√	K($1)IFVQK K($1)K [8:13 87:88]
1075	840	+4	33	18.1	6.4	9.0	390.9924	1560.9479	1560.9472	0.0007	3	√	K($1)IFVQK K($1)KTER [8:13 87:91]
945	1335	+2	30	11.3	10.6	5.1	731.4198	1461.8323	1461.8312	0.0012	2	√	K($1)IFVQK K($1)ATNE
946	1328	+3	10	8.4	4.3	4.5	487.9493	1461.8333	1461.8312	0.0021	2	√	K($1)IFVQK K($1)ATNE [8:13 100:104]
1445	2235	+4	94	24.7	15.5	7.0	454.2489	1813.9737	1813.9820	-0.0084	2	√	GGK($1)HK($1)TGPNLHGLFGR
1446	2242	+3	63	21.9	12.3	16.7	605.3295	1813.9739	1813.9820	-0.0082	2	√	GGK($1)HK($1)TGPNLHGLFGR
1447	2420	+3	36	9.4	9.8	7.3	605.3303	1813.9762	1813.9820	-0.0058	2	√	GGK($1)HK($1)TGPNLHGLFGR
1448	2422	+4	151	21.5	20.5	4.7	454.2502	1813.9789	1813.9820	-0.0031	2	√	GGK($1)HK($1)TGPNLHGLFGR
1449	2501	+3	25	9.4	7.5	5.8	605.3312	1813.9792	1813.9820	-0.0029	2	√	GGK($1)HK($1)TGPNLHGLFGR
1450	2247	+2	32	12.9	9.2	7.3	907.4948	1813.9823	1813.9820	0.0003	2	√	GGK($1)HK($1)TGPNLHGLFGR
1451	2349	+3	60	19.6	11.7	13.1	605.3324	1813.9826	1813.9820	0.0006	2	√	GGK($1)HK($1)TGPNLHGLFGR
1452	2338	+4	95	24.7	15.9	4.7	454.2512	1813.9829	1813.9820	0.0009	2	√	GGK($1)HK($1)TGPNLHGLFGR
1453	2645	+4	82	17.1	15.1	5.4	454.2512	1813.9831	1813.9820	0.0010	2	√	GGK($1)HK($1)TGPNLHGLFGR
1454	2538	+4	66	20.8	15.7	7.0	454.2518	1813.9855	1813.9820	0.0035	2	√	GGK($1)HK($1)TGPNLHGLFGR
1455	2622	+4	81	16.4	14.5	5.4	454.2520	1813.9862	1813.9820	0.0042	2	√	GGK($1)HK($1)TGPNLHGLFGR
1456	2595	+4	87	18.6	15.7	4.7	454.2521	1813.9865	1813.9820	0.0045	2	√	GGK($1)HK($1)TGPNLHGLFGR
1457	2755	+4	99	21.5	18.1	9.7	454.2521	1813.9866	1813.9820	0.0046	2	√	GGK($1)HK($1)TGPNLHGLFGR
1458	2669	+4	74	18.6	14.3	5.4	454.2523	1813.9873	1813.9820	0.0053	2	√	GGK($1)HK($1)TGPNLHGLFGR
1459	2855	+4	18	11.3	7.2	3.4	454.2523	1813.9873	1813.9820	0.0053	2	√	GGK($1)HK($1)TGPNLHGLFGR
1460	2658	+4	74	17.8	14.8	7.8	454.2526	1813.9885	1813.9820	0.0065	2	√	GGK($1)HK($1)TGPNLHGLFGR
1463	2439	+3	51	13.5	10.6	6.5	605.6622	1814.9721	1814.9854	-0.0133	2	√	*GGK($1)HK($1)TGPNLHGLFGR
1464	2436	+4	136	22.3	19.5	7.0	454.4988	1814.9733	1814.9854	-0.0121	2	√	*GGK($1)HK($1)TGPNLHGLFGR [23:38]
298	2890	+2	96	11.5	13.3	9.5	482.7704	964.5336	964.5350	-0.0014	0	√	EDLIAYLK
299	2896	+2	98	12.2	13.7	8.1	482.7705	964.5337	964.5350	-0.0012	0	√	EDLIAYLK
300	2970	+2	93	14.4	13.5	10.9	482.7707	964.5340	964.5350	-0.0009	0	√	EDLIAYLK
301	3348	+2	50	12.7	12.4	8.1	482.7707	964.5340	964.5350	-0.0009	0	√	EDLIAYLK
302	2778	+2	82	9.5	13.5	5.9	482.7710	964.5347	964.5350	-0.0003	0	√	EDLIAYLK
303	2877	+2	97	15.2	13.0	9.5	482.7712	964.5351	964.5350	0.0002	0	√	EDLIAYLK
304	3089	+2	59	10.2	12.6	5.9	482.7713	964.5353	964.5350	0.0003	0	√	EDLIAYLK
305	2978	+2	103	15.2	14.3	9.5	482.7714	964.5355	964.5350	0.0005	0	√	EDLIAYLK
306	2641	+2	52	10.9	11.2	7.0	482.7715	964.5357	964.5350	0.0007	0	√	EDLIAYLK
307	3065	+2	68	10.9	13.4	5.9	482.7722	964.5371	964.5350	0.0021	0	√	EDLIAYLK
308	3307	+2	13	9.5	6.6	4.9	482.7727	964.5381	964.5350	0.0032	0	√	EDLIAYLK
309	3315	+2	34	8.9	10.2	5.9	482.7753	964.5433	964.5350	0.0083	0	√	EDLIAYLK [92:99]
498	2267	+2	35	13.7	5.3	9.3	546.8167	1092.6260	1092.6299	-0.0039	1	√	EDLIAYLKK
499	2273	+3	65	18.9	8.5	10.6	364.8802	1092.6261	1092.6299	-0.0038	1	√	EDLIAYLKK
501	2467	+2	12	5.8	5.9	2.2	546.8215	1092.6358	1092.6299	0.0059	1	√	EDLIAYLKK
744	3207	+2	42	7.0	12.8	4.1	624.3703	1247.7333	1247.7246	0.0088	1	√	EDLIAYLKK + bs32(8)
745	3201	+2	53	8.7	14.9	4.1	624.3715	1247.7356	1247.7246	0.0111	1	√	EDLIAYLKK + bs32(8)
746	3433	+2	74	11.9	18.6	6.9	624.8604	1248.7135	1248.7086	0.0050	1	√	EDLIAYLKK + bs31(8) [92:100]

Basic information about the protein match: theoretical mass, scores, protein description, sequence, sequence coverage, tag coverage. The color tags of the protein sequence will be explained in "**Sequence Color Tag**" Section.

List of peptide matches for the protein. The peptide matches are grouped into blocks by their sequences. The blocks of peptide matches are sorted according to their positions in the protein.

The link in the front of each peptide match is the link to the detailed **Peptide View** for that match. The column names for the peptide matches will be explained in "**Peptide View**" Section. The color tags of peptide sequences will be explained in "**Sequence Color Tag**" Section.

These indicate the positions of the peptides in the protein sequence. If a peptide has more than one chain due to cross-links or disulfide bonds, the positions of all chains will be indicated

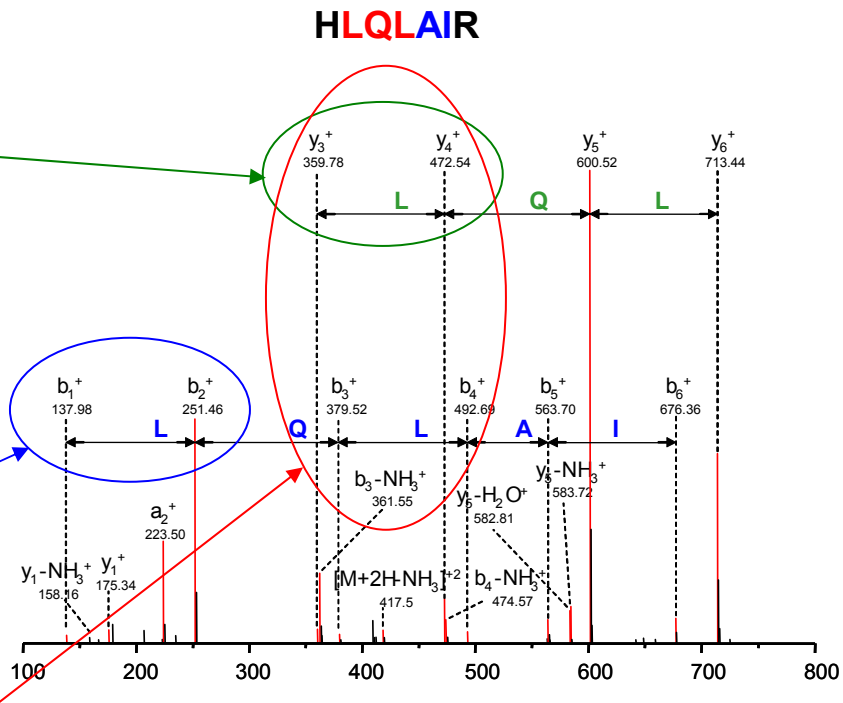
Sequence Color Tag 1: Peptides

Three color tags, i.e. **green**, **blue**, and **red** tags, are used in peptide sequences.

Green tag: In a peptide sequence, an amino acid residue tagged in **green** has a pair of consecutive **y** ions whose mass difference equals the mass of the amino acid residue.

Blue tag: In a peptide sequence, an amino acid residue tagged in **blue** has a pair of consecutive **b** ions whose mass difference equals the mass of the amino acid residue.

Red tag: In a peptide sequence, an amino acid residue tagged in **red** has both pairs of consecutive **y** and **b** ions whose mass differences equal the mass of the amino acid residue, i.e.
red tag = **green** tag + **blue** tag.



In the peptide match above, H**LQLA**IR

H	L	Q	L	A	I	R
No tag	**b**+**y** tags	**b**+**y** tags	**b**+**y** tags	**b** tag	**b** tag	No tag

Note: The color tags of a peptide match can be used as a direct visual indication of the quality of that match. More colored tags are better.

Sequence Color Tag 2: Proteins

```
Sequence:
 001 GDVEKGKKIF VQKCAQCHTV EKGGKHKTGP NLHGLFGRKT GQAPGFTYTD ANKNKGITWK EETLMEYLEN PKKYIPGTKM IFAGIKKKTE REDLIAYLKK  100
 101 ATNE                                                                                                          104

Sequence Coverage: 86%
Sequence Tag Coverage: 84%
```

Four color tags, i.e. **green**, **blue**, **red**, and **yellow** tags, are used in protein sequences.

An amino acid residue tagged in color, i.e. **green**, **blue**, **red**, or **yellow**, in a protein sequence is covered by one or more peptide matches of the protein.

Amino acid residues in black are not covered by any peptide matches of the protein.

1) **Green** tag: At least one peptide match of the protein has a **green** tag for the amino acid residue.

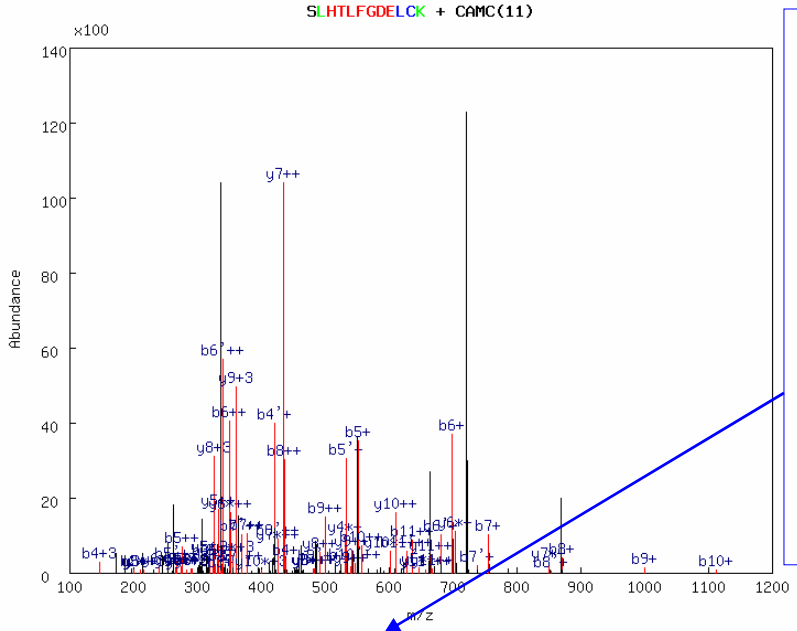
2) **Blue** tag: At least one peptide match of the protein has a **blue** tag for the amino acid residue.

3) **Red** tag: At least one peptide match of the protein has a **green** tag and at least one peptide match of the protein has a **blue** tag for the amino acid residue, i.e. **red** tag = **green** tag + **blue** tag.

4) **Yellow** tag: No peptide matches of the protein have any color tags for the amino acid residue.

Sequence Coverage: Coverage of all four color tags.
Sequence Tag Coverage: Coverage of **green**, **blue**, and **red** tags.
Tag coverage is a better and more robust indication of the protein match quality than sequence coverage.

Peptide View 1 – Details of A Peptide Match



Index: Unique ID for a peptide match, no specific meaning.
Scan#: Scan number of the spectrum used to locate the spectrum in the original RAW file.
Charge: Charge state of the precursor peptide ion.
Score: Empirical score of the match. Not a good standard to evaluate a peptide match.
pp, pp2: Two pp scores based on two statistical models. Values bigger than 6.0 will be highlighted in blue. Big maximum value of pp and pp2 indicates a good match. The pp scores should be used along with pp_{tag} score.
pp_{tag}: A statistical score that evaluates the match based on its color tags. A better score indicates better match quality.

Index	scan#	charge	score	pp	pp2	pp_{tag}	m/z	MW(obs)	MW	delta	miss	Unique	sequence + modifications
633	1842	+3	41	22.7	10.0	17.1	474.2320	1420.6813	1420.6971	-0.0157	0	√	*SLHTLFGDELCK + CAMC(11)

#	b'^{+3}	b^{*+3}	b^{+3}	b'^{++}	b^{*++}	b^{++}	b'^{+}	b^{*+}	b^{+}	seq	y'^{+3}	y^{*+3}	y^{+3}	y'^{++}	y^{*++}	y^{++}	y'^{+}	y^{*+}	y^{+}	#
1	24.01		30.02	35.52		44.52	70.03		88.04	S	467.90	468.23	473.90	701.35	701.84	710.35	1401.68	1402.67	1419.69	M
2	61.71		67.71	92.06		101.07	183.11		201.12	L	438.89	439.22	444.89	657.83	658.32	666.83	1314.65	1315.64	1332.66	11
3	107.40		113.40	160.59		169.59	320.17		338.18	H	401.19	401.52	407.20	601.29	601.78	610.29	1201.57	1202.55	1219.58	10
4	141.08		147.08	211.11		220.12	421.22		439.23	T	355.51	355.84	361.51	532.76	533.25	541.76	1064.51	1065.49	1082.52	9
5	178.77		184.78	267.66		276.66	534.30		552.31	L	321.82	322.15	327.83	482.23	482.73	491.24	963.46	964.44	981.47	8
6	227.80		233.80	341.19		350.19	681.37		699.38	F	284.13	284.46	290.13	425.69	426.18	434.70	850.38	851.36	868.39	7
7	246.80		252.81	369.70		378.71	738.39		756.40	G	235.11	235.44	241.11	352.16	352.65	361.16	703.31	704.29	721.32	6
8	285.14		291.15	427.21		436.22	853.42		871.43	D	216.10	216.43	222.10	323.65	324.14	332.65	646.29	647.27	664.30	5
9	328.16		334.16	491.74		500.74	982.46		1000.47	E	177.76	178.09	183.76	266.13	266.63	275.14	531.26	532.24	549.27	4
10	365.85		371.86	548.28		557.28	1095.55		1113.56	L	134.74	135.07	140.75	201.61	202.10	210.62	402.22	403.20	420.23	3
11	419.20		425.20	628.29		637.30	1255.58		1273.59	C	97.05	97.38	103.05	145.07	145.56	154.08	289.13	290.12	307.14	2
										K	43.71	44.03	49.71	65.05	65.55	74.06	129.10	130.09	147.11	1

m/z: Observed *m/z* value of the precursor peptide ion.
MW(obs), MW: Observed and calculated masses of $[M+H]^+$ for the peptide.
Delta:delta = MW(obs) - MW.
Miss: missed cleavage of the peptide
Unique: This indicates whether the peptide only belongs to one protein or not.
Sequence+modifications: peptide sequence along with its modifications.

Spectral Info:

Scan#	t_R(min)	t_R(Pred)	Conf. t_R	Peak Area
1842	26.01	28.07	73.45%	168104.23

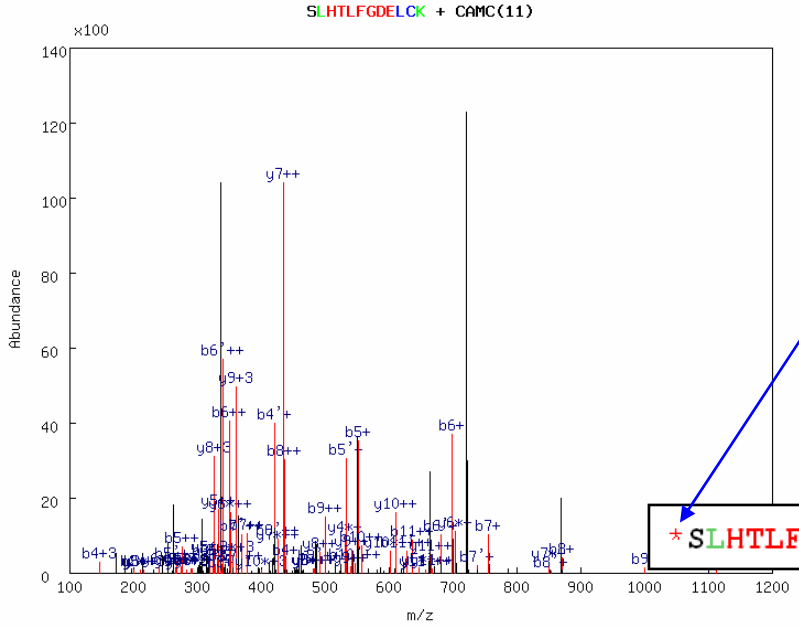
All possible peptide matches for this spectrum

633 1842 +3 41 22.7 10.0 17.1 474.2320 1420.6813 1420.6971 -0.0157 0 √ *SLHTLFGDELCK + CAMC(11)

The peptide is from:

hit1 gi|1351907|Serum albumin precursor (Allergen Bos d 6) (BSA)

Peptide View 1 – Details of A Peptide Match (Con't)



Index scan# charge score pp pp2 pptag m/z MW(obs) MW delta miss Unique sequence + modifications

633 1842 +3 41 22.7 10.0 17.1 474.2320 1420.6813 1420.6971 -0.0157 0 √ *SLHTLFGDELCK + CAMC(11)

#	b'+3	b*+3	b+3	b'++	b*++	b++	b'+	b*+	b+	seq	y'+3	y*+3	y+3	y'++	y*++	y++	y'+	y*+	y+	#
1	24.01		30.02	35.52		44.52	70.03		88.04	S	467.90	468.23	473.90	701.35	701.84	710.35	1401.68	1402.67	1419.69	M
2	61.71		67.71	92.06		101.07	183.11		201.12	L	438.89	439.22	444.89	657.83	658.32	666.83	1314.65	1315.64	1332.66	11
3	107.40		113.40	160.59		169.59	320.17		338.18	H	401.19	401.52	407.20	601.29	601.78	610.29	1201.57	1202.55	1219.58	10
4	141.08		147.08	211.11		220.12	421.22		439.23	T	355.51	355.84	361.51	532.76	533.25	541.76	1064.51	1065.49	1082.52	9
5	178.77		184.78	267.66		276.66	534.30		552.31	L	321.82	322.15	327.83	482.23	482.73	491.24	963.46	964.44	981.47	8
6	227.80		233.80	341.19		350.19	681.37		699.38	F	284.13	284.46	290.13	425.69	426.18	434.70	850.38	851.36	868.39	7
7	246.80		252.81	369.70		378.71	738.39		756.40	G	235.11	235.44	241.11	352.16	352.65	361.16	703.31	704.29	721.32	6
8	285.14		291.15	427.21		436.22	853.42		871.43	D	216.10	216.43	222.10	323.65	324.14	332.65	646.29	647.27	664.30	5
9	328.16		334.16	491.74		500.74	982.46		1000.47	E	177.76	178.09	183.76	266.13	266.63	275.14	531.26	532.24	549.27	4
10	365.85		371.86	548.28		557.28	1095.55		1113.56	L	134.74	135.07	140.75	201.61	202.10	210.62	402.22	403.20	420.23	3
11	419.20		425.20	628.29		637.30	1255.58		1273.59	C	97.05	97.38	103.05	145.07	145.56	154.08	289.13	290.12	307.14	2
										K	43.71	44.03	49.71	65.05	65.55	74.06	129.10	130.09	147.11	1

Spectral Info:

Scan# tR(min) tR(Pred) Conf. tR Peak Area

1842 26.01 28.07 73.45% 168104.23

All possible peptide matches for this spectrum

633 1842 +3 41 22.7 10.0 17.1 474.2320 1420.6813 1420.6971 -0.0157 0 √ *SLHTLFGDELCK + CAMC(11)

The peptide is from:

hit1 gi|1351907|Serum albumin precursor (Allergen Bos d 6) (BSA)

* in front of a peptide match indicates that the precursor peptide ion is not monoisotopic and has one ^{13}C in it.

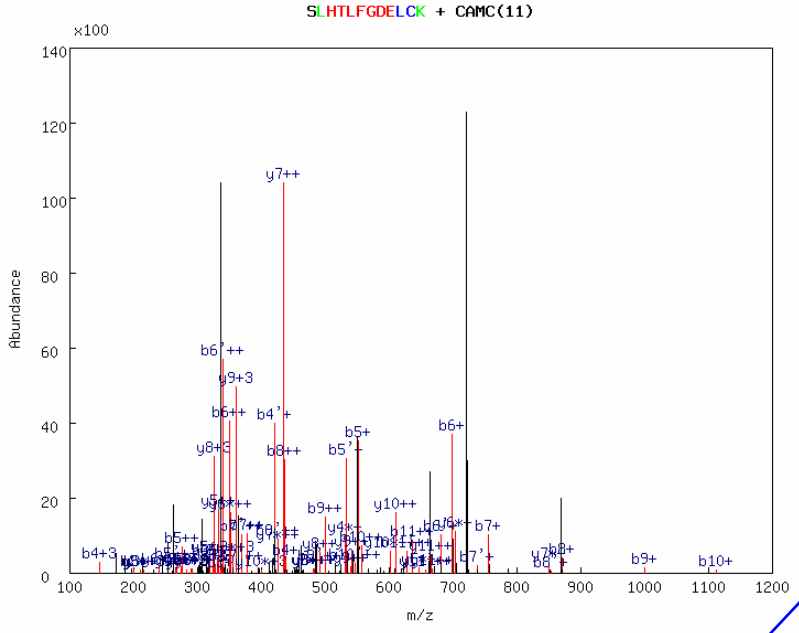
Color tags of the peptide match. See "**Sequence Color Tag**" section for more details.

Modification of the peptide match. Type of the modification is indicated by the label. Location of the modification is indicated by the number in the bracket. The 11th amino acid residue of this peptide is modified by CAMC (Carbamidomethyl of Cys). If no location is indicated, the modification is on N-terminus or C-terminus of the peptide.

Theoretical product ions from the fragmentation of the precursor peptide ions. The b/y ions are considered for CID fragmentation.

Product ions due to neutral losses are labeled as follows: ~ (loss of phosphate moiety on S and T), ^ (loss of phosphate moiety on Y), * (loss of ammonia), and ' (loss of water).

Peptide View 1 – Details of A Peptide Match (Con't)



This section contains information of the experimental spectrum and is only available when mzXML is provided during search.

Scan#: Scan number of the spectrum used to locate the spectrum in the original RAW file.

t_R: Observed retention time of the spectrum in minutes.

t_R(Pred): Predicted retention time of the peptide match for this spectrum.

Conf. t_R: Confidence score based the observed and predicted retention times. If this confidence score < 1%, you can reject this match as a false match and the chance that the match is a falsely rejected true match is < 1%.

Peak Area: The peak area of the LC elution profile of the peptide.

Index	scan#	charge	score	pp	pp2	pptag	m/z	MW(obs)	MW	delta	miss	Unique	sequence + modifications
633	1842	+3	41	22.7	10.0	17.1	474.2320	1420.6813	1420.6971	-0.0157	0	√	*SLHTLFGDELCK + CAM

#	b'+3	b*+3	b+3	b'++	b*++	b++	b'+	b*+	b+	seq	y'+3	y*+3	y+3	y'++	y*++	y++	y'+	y*+	y+	#
1	24.01		30.02	35.52		44.52	70.03		88.04	S	467.90	468.23	473.90	701.35	701.84	710.35	1401.68	1402.67	1419.69	M
2	61.71		67.71	92.06		101.07	183.11		201.12	L	438.89	439.22	444.89	657.83	658.32	666.83	1314.65	1315.64	1332.66	11
3	107.40		113.40	160.59		169.59	320.17		338.18	H	401.19	401.52	407.20	601.29	601.78	610.29	1201.57	1202.55	1219.58	10
4	141.08		147.08	211.11		220.12	421.22		439.23	T	355.51	355.84	361.51	532.76	533.25	541.76	1064.51	1065.49	1082.52	9
5	178.77		184.78	267.66		276.66	534.30		552.31	L	321.82	322.15	327.83	482.23	482.73	491.24	963.46	964.44	981.47	8
6	227.80		233.80	341.19		350.19	681.37		699.38	F	284.13	284.46	290.13	425.69	426.18	434.70	850.38	851.36	868.39	7
7	246.80		252.81	369.70		378.71	738.39		756.40	G	235.11	235.44	241.11	352.16	352.65	361.16	703.31	704.29	721.32	6
8	285.14		291.15	427.21		436.22	853.42		871.43	D	216.10	216.43	222.10	323.65	324.14	332.65	646.29	647.27	664.30	5
9	328.16		334.16	491.74		500.74	982.46		1000.47	E	177.76	178.09	183.76	266.13	266.63	275.14	531.26	532.24	549.27	4
10	365.85		371.86	548.28		557.28	1095.55		1113.56	L	134.74	135.07	140.75	201.61	202.10	210.62	402.22	403.20	420.23	3
11	419.20		425.20	628.29		637.30	1255.58		1273.59	C	97.05	97.38	103.05	145.07	145.56	154.08	289.13	290.12	307.14	2
										K	43.71	44.03	49.71	65.05	65.55	74.06	129.10	130.09	147.11	1

This section contains a list of all candidate peptide matches for this experimental spectrum. It is very useful when you have multiple candidate peptide matches for one experimental spectrum with close scores. Clicking on the link in the front of each match will direct you to that peptide match.

This section contains all the protein matches that have the peptide match. Clicking on the link in the front of each protein will direct you to that protein match.

Spectral Info:

Scan#	t_R(min)	t_R(Pred)	Conf. t_R	Peak Area
1842	26.01	28.07	73.45%	168104.23

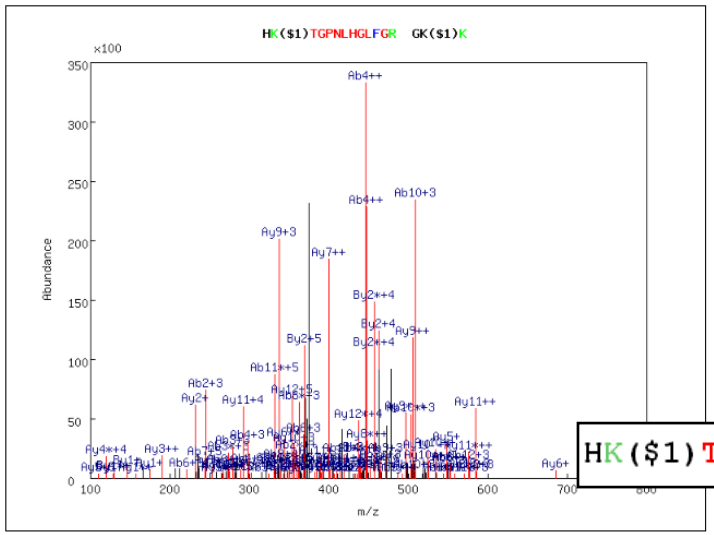
All possible peptide matches for this spectrum

633 1842 +3 41 22.7 10.0 17.1 474.2320 1420.6813 1420.6971 -0.0157 0 √ *SLHTLFGDELCK + CAMC(11)

The peptide is from:

hit1 gi|1351907|Serum albumin precursor (Allergen Bos d 6) (BSA)

Peptide View 2 –
Details of A Cross-Linked Peptide Match

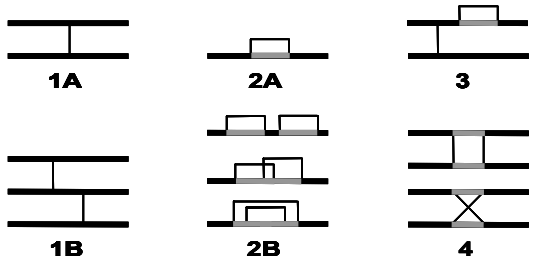


HK($1)TGPNLHGLFGR GK($1)K

Index scan# charge score pp pp_2 pp_{rag} m/z MW(obs) MW delta miss Unique sequence + modifications

1547 1740 +5 53 19.0 11.1 9.8 381.4182 1903.0621 1903.0661 -0.0040 2 √ HK($1)TGPNLHGLFGR GK($1)K

Cross-Linked or disulfide-linked peptides can have multiple chains and different shapes as follows



This example cross-linked peptide match is of type 1A. Different chains of the peptide are separated by spaces. Link sites are labeled by "$*i*" (*i* = 1,2…). Two sites with the same label form a cross-link or disulfide bond.

chainA :

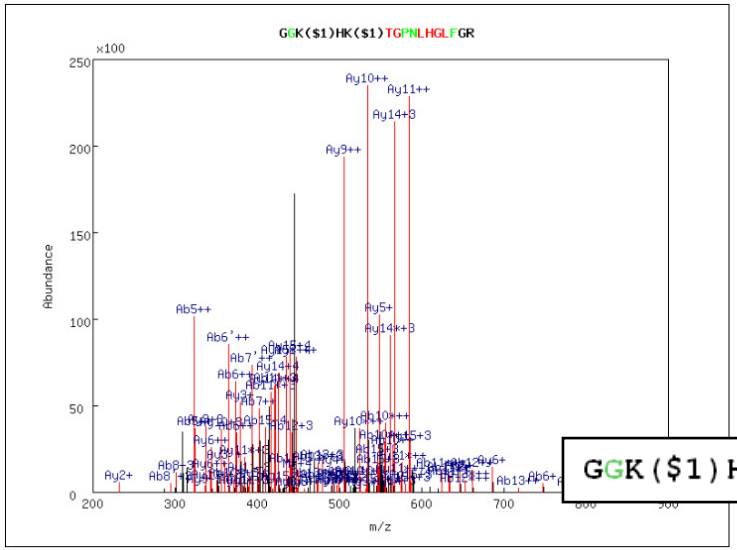
#	b'+5	b*+5	b+5	b'+4	b*+4	b+4	b'+3	b*+3	b+3	b'++	b*++	b++	b'+	b*+	b+	seq	y'+5	y*+5	y+5	y'+4	y*+4	y+4	y'+3	y*+3	y+3	y'++	y*++	y++	y'+	y*+	y+	#
1			28.42			35.27			46.69			69.54			138.07	H	377.82	378.01	381.42	472.02	472.27	476.52	629.02	629.35	635.03	943.03	943.53	952.04	1885.06	1886.04	1903.07	M	
2	144.29	144.49	147.90	180.12	180.36	184.62	239.82	240.15	245.82	359.22	359.72	368.23	717.44	718.42	735.45	K	350.41	350.60	354.01	437.75	438.00	442.26	583.34	583.67	589.34	874.50	874.					
3	164.50	164.70	168.11	205.38	205.62	209.88	273.50	273.83	279.50	409.75	410.24	418.75	818.49	819.47	836.50	T	230.93	231.12	234.53	288.41	288.65	292.91	384.21	384.54	390.21	575.81	576.					
4	175.91	176.10	179.51	219.63	219.88	224.14	292.51	292.84	298.51	438.26	438.75	447.26	875.51	876.49	893.52	G	210.72	210.92	214.32	263.15	263.39	267.65	350.53	350.85	356.53	525.29	525.					
5	195.32	195.52	198.92	243.90	244.14	248.40	324.86	325.19	330.86	486.78	487.28	495.79	972.56	973.55	990.57	P	199.31	199.51	202.92	248.89	249.14	253.39	331.52	331.85	337.52	496.77	497.					
6	218.13	218.32	221.73	272.41	272.65	276.91	362.87	363.20	368.88	543.81	544.30	552.81	1086.61	1087.59	1104.62	N	179.90	180.10	183.51	224.63	224.87	229.13	299.17	299.50	305.17	448.25	448.					
7	240.74	240.94	244.35	300.68	300.92	305.18	400.57	400.90	406.57	600.35	600.84	609.35	1199.69	1200.67	1217.70	L	157.10	157.29	160.70	196.12	196.36	200.62	261.15	261.48	267.16	391.23	391.					
8	268.16	268.35	271.76	334.94	335.19	339.45	446.25	446.58	452.26	668.88	669.37	677.88	1336.75	1337.73	1354.76	H	134.48	134.68	138.08	167.85	168.09	172.35	223.46	223.79	229.46	334.68	335.					
9	279.56	279.76	283.16	349.20	349.44	353.70	465.26	465.59	471.27	697.39	697.88	706.39	1393.77	1394.75	1411.78	G	107.07	107.26	110.67	133.58	133.83	138.08	177.77	178.10	183.78	266.16	266.					
10	302.18	302.37	305.78	377.47	377.71	381.97	502.96	503.28	508.96	753.93	754.42	762.94	1506.85	1507.84	1524.86	L	95.66	95.86	99.26	119.33	119.57	123.83	158.77	159.09	164.77	237.64	238.					
11	331.59	331.79	335.19	414.24	414.48	418.74	551.98	552.31	557.98	827.46	827.96	836.47	1653.92	1654.91	1671.93	F	73.05	73.24	76.65	91.06	91.30	95.56	121.07	121.40	127.07	181.10	181.					
12	342.99	343.19	346.60	428.49	428.74	432.99	570.99	571.31	576.99	855.98	856.47	864.98	1710.94	1711.93	1728.95	G	43.63	43.83	47.23	54.29	54.53	58.79	72.05	72.38	78.05	107.57	108.					
																R	32.23	32.42	35.83	40.03	40.28	44.54	53.04	53.37	59.04	79.06	79.					

Peptides with multiple chains can have product ions created from the ruptures taking place on all the chains. This example peptide has two chains and the product ions are listed in two tables for the two chains.

chainB :

#	b'+5	b*+5	b+5	b'+4	b*+4	b+4	b'+3	b*+3	b+3	b'++	b*++	b++	b'+	b*+	b+	seq	y'+5	y*+5	y+5	y'+4	y*+4	y+4	y'+3	y*+3	y+3	y'++	y*++	y++	y'+	y*+	y+	#
1			12.41			15.26			20.01			29.52			58.03	G																
2	348.60	348.79	352.20	435.49	435.74	440.00	580.32	580.65	586.33	869.98	870.47	878.98	1738.95	1739.93	1756.96	K	366.41	366.61	370.01	457.76	458.01	462.27	610.02	610.34	616.02	914.52	915.01	923.53	1828.03	1829.02	1846.04	2
																K	26.63	26.82	30.23	33.03	33.28	37.53	43.71	44.03	49.71	65.05	65.55	74.06	129.10	130.09	147.11	1

Peptide View 2 – Details of A Cross-Linked Peptide Match (Con't)



GGK($1)HK($1)TGPNLHGLFGR

This example cross-linked peptide matches is of type 2A.

Peptides with intra-chain cross-links or disulfide bonds will have blind spots where any rupture will not create signature product ions.

Blind spot for b ions.

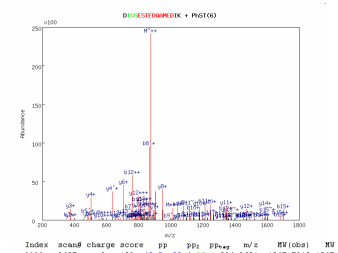
Blind spot for y ions.

Index	scan#	charge	score	pp	pp2	pptag	m/z	MW(obs)	MW	delta	miss	Unique	sequence + modifications
1445	2235	+4	94	24.7	15.5	7.0	454.2489	1813.9737	1813.9820	-0.0084	2	√	GGK($1)HK($1)TGPNLHGLFGR

chainA :

#	b'+4	b*+4	b+4	b'+3	b*+3	b+3	b'++	b*++	b++	b'	b*	b+	seq	y'+4	y*+4	y+4	y'+3	y*+3	y+3	y'++	y*++	y++	y'	y*	y+	
1			15.26			20.01			29.52			58.03	G	449.75	449.99	454.25	599.33	599.66	605.33	898.49	898.98	907.49	1795.97	1796.96	1813.98	
2			29.52			39.02			58.03			115.05	G	435.49	435.74	440.00	580.32	580.65	586.33	869.98	870.47	878.98	1738.95	1739.93	1756.96	
3													L	421.24	421.48	425.74	561.31	561.64	567.32	841.47	841.96	850.47	1681.93	1682.91	1699.94	
4													H													
5			162.35			216.13			323.69			646.37	K													12
6	183.11		187.61	243.81		249.81	365.21		374.21	729.40		747.41	T	288.41	288.65	292.91	384.21	384.54	390.21	576.81	576.30	584.81	1150.61	1151.60	1168.62	11
7	197.36		201.86	262.81		268.82	393.72		402.72	786.43		804.44	G	263.15	263.39	267.65	350.53	350.85	356.53	525.29	525.78	534.29	1049.56	1050.55	1067.57	10
8	221.63		226.13	295.16		301.17	442.24		451.25	883.48		901.49	P	248.89	249.14	253.39	331.52	331.85	337.52	496.77	497.27	505.78	992.54	993.53	1010.55	9
9	250.14	250.38	254.64	333.18	333.51	339.18	499.26	499.76	508.27	997.52	998.51	1015.53	N	224.63	224.87	229.13	299.17	299.50	305.17	448.25	448.74	457.25	895.49	896.47	913.50	8
10	278.41	278.65	282.91	370.87	371.20	376.88	555.81	556.30	564.81	1110.61	1111.59	1128.62	L	196.12	196.36	200.62	261.15	261.48	267.16	391.23	391.72	400.23	781.45	782.43	799.46	7
11	312.67	312.92	317.17	416.56	416.89	422.56	624.34	624.83	633.34	1247.66	1248.65	1265.67	H	167.85	168.09	172.35	223.46	223.79	229.46	334.68	335.18	343.69	668.36	669.35	686.37	6
12	326.93	327.17	331.43	435.57	435.89	441.57	652.85	653.34	661.85	1304.69	1305.67	1322.70	G	133.58	133.83	138.08	177.77	178.10	183.78	266.16	266.65	275.16	531.30	532.29	549.31	5
13	355.20	355.44	359.70	473.26	473.59	479.27	709.39	709.88	718.39	1417.77	1418.75	1435.78	L	119.33	119.57	123.83	158.77	159.09	164.77	237.64	238.14	246.65	474.28	475.27	492.29	4
14	391.97	392.21	396.47	522.28	522.61	528.29	782.92	783.41	791.93	1564.84	1565.82	1582.85	F	91.06	91.30	95.56	121.07	121.40	127.07	181.10	181.59	190.11	361.20	362.18	379.21	3
15	406.22	406.47	410.72	541.29	541.62	547.29	811.43	811.93	820.44	1621.86	1622.84	1639.87	G	54.29	54.53	58.79	72.05	72.38	78.05	107.57	108.06	116.57	214.13	215.11	232.14	2
													R	40.03	40.28	44.54	53.04	53.37	59.04	79.06	79.55	88.06	157.11	158.09	175.12	1

Peptide View 3 – Details of A Peptide Match with MS^3 Data



Index scan# charge score pp ppr ppseq m/z MW(obs) MW delta miss Unique sequence + modifications

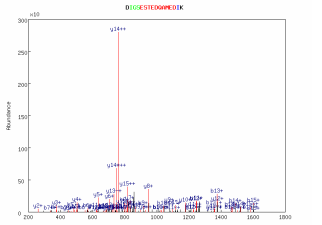
2322 2497 +2 102 42.0 20.4 25.1 924.3691 1847.7310 1847.7252 0.0058 0 √ DIGSESTEDQAMEDIK + PhST(6)

#	b^{++}	b^{+++}	b^{++}	b^{+++}	b^{++}	b^{+++}	b^{+}	b^{++}	b^{++}	b^{+}	seq	y^{+++}	y^{++}	y^{+++}	y^{++}	y^{+++}	y^{+}	y^{++}	y^{+}	y^{0}	#
1			49.52		58.52			98.02		116.03	D	875.38	915.36	915.85	924.37		1749.75	1829.71	1830.70	1847.73	16
2			106.06		115.06			211.11		229.12	I	817.86	857.85	858.34	866.85		1634.72	1714.69	1715.67	1732.70	15
3			134.57		143.57			268.13		286.14	G	761.32	801.31	801.80	810.31		1521.64	1601.60	1602.59	1619.61	14
4			178.08		187.09			355.16		373.17	S	732.81	772.79	773.29	781.80		1464.62	1544.58	1545.57	1562.59	13
5			242.61		251.61			484.20		502.21	E	689.30	729.28	729.77	738.28		1377.58	1457.55	1458.53	1475.56	12
6		286.12	326.10		335.11		571.24	651.20		669.21	S	624.77	664.76	665.25	673.76		1248.54	1328.51	1329.49	1346.52	11
7		336.65	376.63		385.63		672.28	752.25		770.26	T		581.26	581.75	590.26			1161.51	1162.49	1179.52	10
8		401.17	441.15		450.16		801.33	881.29		899.30	E		530.73	531.23	539.74			1060.46	1061.45	1078.47	9
9		458.68	498.66		507.67		916.35	996.32		1014.33	D		466.21	466.71	475.22			931.42	932.40	949.43	8
10		522.71	562.69	563.18	571.70		1044.41	1124.38	1125.36	1142.39	Q		408.70	409.19	417.70			816.39	817.38	834.40	7
11		558.23	598.21	598.70	607.22		1115.45	1195.42	1196.40	1213.43	A		344.67	345.16	353.68			688.33	689.32	706.34	6
12		623.75	663.73	664.22	672.74		1246.49	1326.46	1327.44	1344.47	M		309.15	309.64	318.16			617.30	618.28	635.31	5
13		688.27	728.25	728.74	737.26		1375.53	1455.50	1456.48	1473.51	E		243.63	244.12	252.64			486.26	487.24	504.27	4
14		745.78	785.77	786.26	794.77		1490.56	1570.53	1571.51	1588.54	D		179.11	179.60	188.12			357.21	358.20	375.22	3
15		802.33	842.31	842.80	851.31		1603.64	1683.61	1684.59	1701.62	I		121.60	122.09	130.60			242.19	243.17	260.20	2
16											K		65.05	65.55	74.06			129.10	130.09	147.11	1

Spectral Info:

Scan#	t_R(min)	t_R(Pred)	Conf. t_R	Peak Area
2497	37.85	29.23	9.37%	0.00

MS3 spectrum for DIGSESTEDQAMEDIK(M-++) in the previous MS2 spectrum



Index scan# charge score pp ppr ppseq m/z MW(obs) MW delta miss Unique sequence + modifications

3324 2498 +2 41 22.6 9.2 10.2 875.63 1750.26 1749.75 0.51 - - DIGSESTEDQAMEDIK

#	b^{++}	b^{+++}	b^{++}	b^{+++}	b^{++}	b^{+++}	b^{+}	seq	y^{+++}	y^{+++}	y^{++}	y^{+++}	y^{+}	y^{0}	#
1	49.52		58.52	98.02			116.03	D	866.37	866.86	875.38	1731.74	1732.72	1749.75	16
2	106.06		115.06	211.11			229.12	I	808.86	809.35	817.86	1616.71	1617.69	1634.72	15
3	134.57		143.57	268.13			286.14	G	752.32	752.81	761.32	1503.63	1504.61	1521.64	14
4	178.08		187.09	355.16			373.17	S	723.81	724.30	732.81	1446.61	1447.59	1464.62	13
5	242.61		251.61	484.20			502.21	E	680.29	680.78	689.30	1359.57	1360.56	1377.58	12
6	277.12		286.12	553.23			571.24	S	615.77	616.26	624.77	1230.53	1231.51	1248.54	11
7	327.64		336.65	654.27			672.28	T	581.26	581.75	590.26	1161.51	1162.49	1179.52	10
8	392.16		401.17	783.32			801.33	E	530.73	531.23	539.74	1060.46	1061.45	1078.47	9
9	449.67		458.68	898.34			916.35	D	466.21	466.71	475.22	931.42	932.40	949.43	8
10	513.70	514.20	522.71	1026.40	1027.39		1044.41	Q	408.70	409.19	417.70	816.39	817.38	834.40	7
11	549.22	549.71	558.23	1097.44	1098.42		1115.45	A	344.67	345.16	353.68	688.33	689.32	706.34	6
12	614.74	615.23	623.75	1228.48	1229.46		1246.49	M	309.15	309.64	318.16	617.30	618.28	635.31	5
13	679.26	679.76	688.27	1357.52	1358.51		1375.53	E	243.63	244.12	252.64	486.26	487.24	504.27	4
14	736.78	737.27	745.78	1472.55	1473.53		1490.56	D	179.11	179.60	188.12	357.21	358.20	375.22	3
15	793.32	793.81	802.33	1585.63	1586.62		1603.64	I	121.60	122.09	130.60	242.19	243.17	260.20	2
16								K	65.05	65.55	74.06	129.10	130.09	147.11	1

All possible peptide matches for this spectrum

2322 2497 +2 102 42.0 20.4 25.1 924.3691 1847.7310 1847.7252 0.0058 0 √ DIGSESTEDQAMEDIK + PhST(6)

The peptide is from:

hit1 gi|30794348|ref|NP_851372.1| gi|30794348|ref|NP_851372.1| casein alpha s1 [Bos taurus]

For peptide matches with MS^3 data, the MS^2 spectrum and all product MS^3 spectra (one or more) for that MS^2 spectrum will be shown in the peptide view of the peptide match. Those spectra are displayed hierarchically.

Search of tandem MS data with MS^3 data in MassMatrix is a easy job. Everything is automated. ***Please provide MassMatrix your MS data of mzXML format if you want to search MS^3 data.*** MS^3 will be automatically searched hierarchically by MassMatrix and displayed in the results. No additional parameters need to be specified and no additional steps need to be performed after the search.

Peptide Match Summary

Peptide match summary lists all the peptide matches in a search regardless of the proteins that they belong to. The matches are grouped into blocks according to their sequences. The blocks of matches are sorted according to their qualities. Best quality peptides are shown on the top of the list.

Peptide Match Summary

Index	scan#	charge	score	pp	pp_2	pp_{tag}	m/z	MW(obs)	MW	delta	miss	Unique	sequence + modifications
1445	2235	+4	94	24.7	15.5	7.0	454.2489	1813.9737	1813.9820	-0.0084	2	√	GGK($1)HK($1)TGPNLHGLFGR
1446	2242	+3	63	21.9	12.3	16.7	605.3295	1813.9739	1813.9820	-0.0082	2	√	GGK($1)HK($1)TGPNLHGLFGR
1447	2420	+3	36	9.4	9.8	7.3	605.3303	1813.9762	1813.9820	-0.0058	2	√	GGK($1)HK($1)TGPNLHGLFGR
1448	2422	+4	151	21.5	20.5	4.7	454.2502	1813.9789	1813.9820	-0.0031	2	√	GGK($1)HK($1)TGPNLHGLFGR
1449	2501	+3	25	9.4	7.5	5.8	605.3312	1813.9792	1813.9820	-0.0029	2	√	GGK($1)HK($1)TGPNLHGLFGR
1450	2247	+2	32	12.9	9.2	7.3	907.4948	1813.9823	1813.9820	0.0003	2	√	GGK($1)HK($1)TGPNLHGLFGR
1451	2349	+3	60	19.6	11.7	13.1	605.3324	1813.9826	1813.9820	0.0006	2	√	GGK($1)HK($1)TGPNLHGLFGR
1452	2338	+4	95	24.7	15.9	4.7	454.2512	1813.9829	1813.9820	0.0009	2	√	GGK($1)HK($1)TGPNLHGLFGR
1453	2645	+4	82	17.1	15.1	5.4	454.2512	1813.9831	1813.9820	0.0010	2	√	GGK($1)HK($1)TGPNLHGLFGR
1454	2538	+4	66	20.8	15.7	7.0	454.2518	1813.9855	1813.9820	0.0035	2	√	GGK($1)HK($1)TGPNLHGLFGR
1455	2622	+4	81	16.4	14.5	5.4	454.2520	1813.9862	1813.9820	0.0042	2	√	GGK($1)HK($1)TGPNLHGLFGR
1456	2595	+4	87	18.6	15.7	4.7	454.2521	1813.9865	1813.9820	0.0045	2	√	GGK($1)HK($1)TGPNLHGLFGR
1457	2755	+4	99	21.5	18.1	9.7	454.2521	1813.9866	1813.9820	0.0046	2	√	GGK($1)HK($1)TGPNLHGLFGR
1458	2669	+4	74	18.6	14.3	5.4	454.2523	1813.9873	1813.9820	0.0053	2	√	GGK($1)HK($1)TGPNLHGLFGR
1459	2855	+4	18	11.3	7.2	3.4	454.2523	1813.9873	1813.9820	0.0053	2	√	GGK($1)HK($1)TGPNLHGLFGR
1460	2658	+4	74	17.8	14.8	7.8	454.2526	1813.9885	1813.9820	0.0065	2	√	GGK($1)HK($1)TGPNLHGLFGR
1463	2439	+3	51	13.5	10.6	6.5	605.6622	1814.9721	1814.9854	-0.0133	2	√	*GGK($1)HK($1)TGPNLHGLFGR
1464	2436	+4	136	22.3	19.5	7.0	454.4988	1814.9733	1814.9854	-0.0121	2	√	*GGK($1)HK($1)TGPNLHGLFGR
1117	1615	+3	29	16.8	7.6	11.4	533.5978	1598.7788	1598.7809	-0.0021	1	√	KTGQAPGFTYTDANK
1118	999	+3	32	30.6	7.0	15.4	533.5984	1598.7808	1598.7809	-0.0001	1	√	KTGQAPGFTYTDANK
1119	1529	+3	24	15.5	6.3	9.6	533.5984	1598.7808	1598.7809	-0.0001	1	√	KTGQAPGFTYTDANK
1120	1148	+3	28	27.2	6.9	12.3	533.5986	1598.7812	1598.7809	0.0003	1	√	KTGQAPGFTYTDANK
1121	1450	+3	20	15.5	5.6	9.6	533.5986	1598.7812	1598.7809	0.0003	1	√	KTGQAPGFTYTDANK
1122	1380	+3	25	19.6	5.7	14.4	533.5988	1598.7817	1598.7809	0.0008	1	√	KTGQAPGFTYTDANK
1123	1268	+3	25	21.0	6.2	9.6	533.5988	1598.7819	1598.7809	0.0010	1	√	KTGQAPGFTYTDANK
1124	1144	+2	44	18.9	9.5	11.4	799.8949	1598.7825	1598.7809	0.0016	1	√	KTGQAPGFTYTDANK
1125	1252	+2	33	11.4	8.9	5.7	799.8950	1598.7826	1598.7809	0.0017	1	√	KTGQAPGFTYTDANK
1126	1966	+3	19	11.9	6.1	10.5	533.5994	1598.7835	1598.7809	0.0026	1	√	KTGQAPGFTYTDANK
1127	1838	+3	22	5.6	5.2	5.0	533.5996	1598.7843	1598.7809	0.0034	1	√	KTGQAPGFTYTDANK
1128	1061	+2	28	10.3	7.0	10.5	799.8959	1598.7845	1598.7809	0.0036	1	√	KTGQAPGFTYTDANK
1130	1164	+3	33	24.0	6.3	12.3	533.9296	1599.7743	1599.7843	-0.0100	1	√	*KTGQAPGFTYTDANK
1131	1159	+2	35	14.9	7.7	7.9	800.3924	1599.7775	1599.7843	-0.0068	1	√	*KTGQAPGFTYTDANK
1396	1725	+2	41	14.8	9.0	11.5	877.4426	1753.8780	1753.8755	0.0024	1	√	KTGQAPGFTYTDANK + bs32(1)
1397	1998	+2	43	14.8	8.9	10.5	877.9364	1754.8655	1754.8596	0.0060	1	√	KTGQAPGFTYTDANK + bs31(1)
1674	2945	+2	127	20.6	18.5	12.0	1105.0648	2209.1224	2209.1209	0.0014	2	√	GITWKEETLMEYLENPKK
1675	3017	+4	25	20.2	4.3	11.3	553.0362	2209.1229	2209.1209	0.0020	2	√	GITWKEETLMEYLENPKK
1676	2932	+3	59	18.5	12.3	17.0	737.0461	2209.1239	2209.1209	0.0029	2	√	GITWKEETLMEYLENPKK
1677	2930	+4	25	10.3	4.7	5.8	553.0364	2209.1239	2209.1209	0.0030	2	√	GITWKEETLMEYLENPKK
1678	3020	+3	64	19.9	12.3	12.9	737.0464	2209.1246	2209.1209	0.0037	2	√	GITWKEETLMEYLENPKK
1679	3107	+4	24	14.9	4.4	9.6	553.0367	2209.1249	2209.1209	0.0040	2	√	GITWKEETLMEYLENPKK
1680	3109	+3	55	15.2	11.6	7.1	737.0482	2209.1299	2209.1209	0.0090	2	√	GITWKEETLMEYLENPKK

Spec Summary

Spec summary lists all the peptide matches just as “Peptide Match Summary” does. However, the matches are grouped into blocks according to their experimental spectra. The blocks of matches are sorted according to their peptide masses. Peptide matches with smallest masses are on the top of the list.

Spec Summary

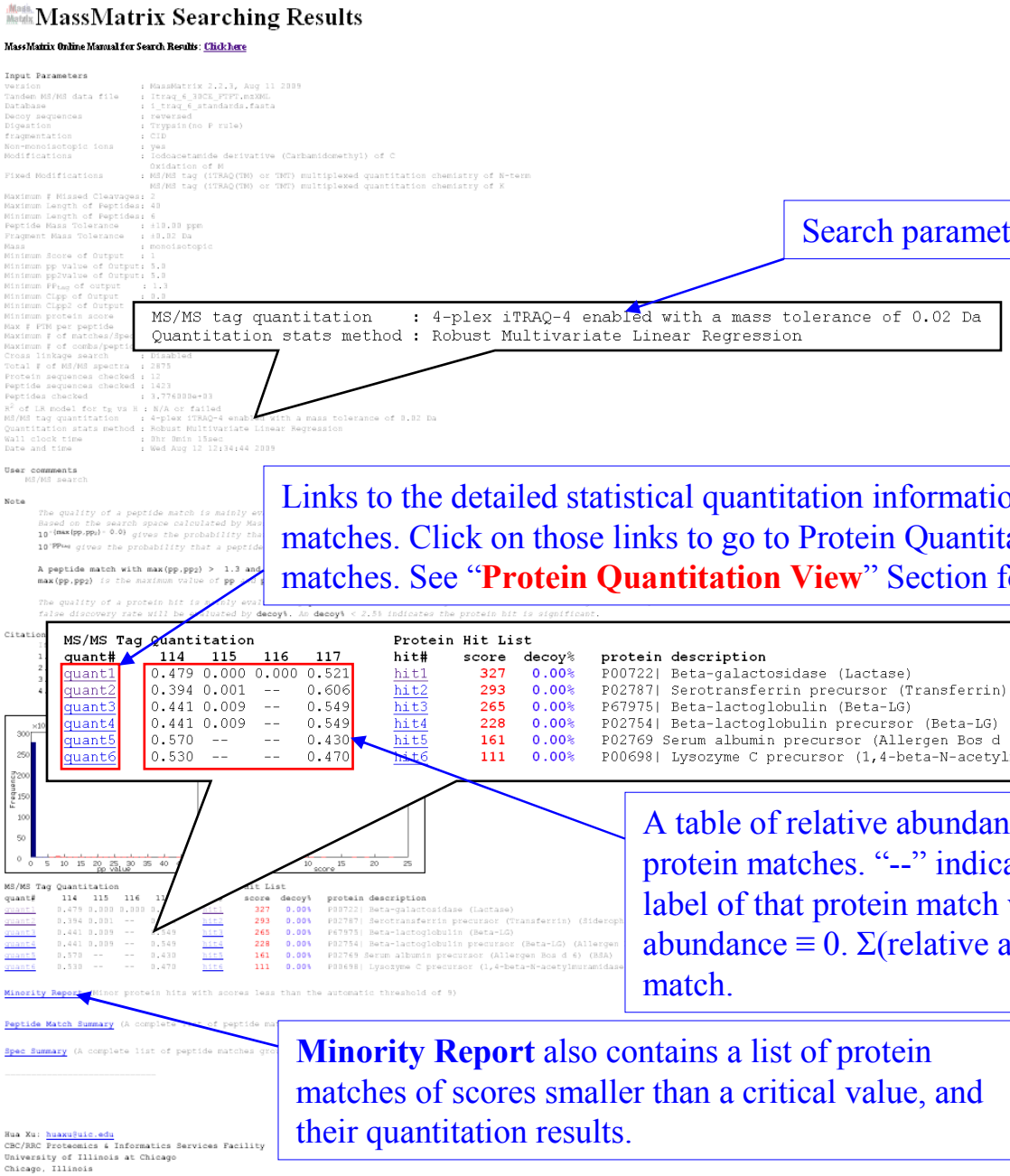
Index	scan#	charge	score	pp	pp_2	pp_{tag}	m/z	MW(obs)	MW	delta	miss	Unique	sequence + modifications
14	707	+2	58	13.2	7.2	2.5	339.6943	678.3813	678.3821	-0.0008	0	√	YIPGTK
15	629	+2	43	9.5	8.3	1.8	339.6947	678.3821	678.3821	-0.0000	0	√	YIPGTK
20	2584	+2	63	8.8	13.1	2.4	345.6946	690.3819	690.3781	0.0038	1	√	ENTAKK
21	2587	+2	65	10.9	12.1	7.2	345.6951	690.3830	690.3781	0.0049	1	√	ENTAKK
81	705	+2	26	10.2	7.3	4.4	381.7464	762.4855	762.4872	-0.0017	1	√	KIFVQK
82	576	+2	36	8.8	8.0	8.2	381.7470	762.4867	762.4872	-0.0005	1	√	KIFVQK
83	723	+2	19	7.5	7.1	5.5	381.7472	762.4871	762.4872	-0.0001	1	√	KIFVQK
101	2216	+2	12	13.5	5.7	10.0	390.2263	779.4453	779.4484	-0.0031	0	√	MIFAGIK
133	736	+2	39	8.9	10.8	4.6	403.7418	806.4762	806.4771	-0.0008	1	√	KYIPGTK
134	628	+2	34	15.3	8.6	9.3	403.7422	806.4771	806.4771	0.0000	1	√	KYIPGTK
135	545	+2	34	11.6	6.7	7.9	403.7422	806.4772	806.4771	0.0001	1	√	KYIPGTK
136	1044	+2	34	10.2	11.5	2.2	403.7433	806.4794	806.4771	0.0023	1	√	KYIPGTK
137	1051	+2	25	10.9	9.3	3.7	403.7439	806.4806	806.4771	0.0035	1	√	KYIPGTK
178	884	+2	27	12.6	9.1	5.2	423.7455	846.4838	846.4832	0.0006	1	√	NKGITWK
227	1788	+2	19	7.1	7.3	2.5	454.2748	907.5423	907.5434	-0.0010	1	√	KIGAFIMK
229	1572	+2	17	8.7	8.5	8.8	454.2773	907.5473	907.5434	0.0039	1	√	MIFAGIKK
245	1668	+2	55	5.9	17.8	4.4	459.2935	917.5798	917.5819	-0.0020	1	√	KIFVQK + bs32(1)
246	2044	+2	36	9.3	13.9	7.1	459.7860	918.5648	918.5659	-0.0011	1	√	KIFVQK + bs31(1)
292	1558	+2	32	6.6	8.7	7.2	481.2889	961.5705	961.5717	-0.0012	1	√	KYIPGTK + bs32(1)
295	1953	+2	53	12.1	11.7	8.8	481.7818	962.5563	962.5557	0.0006	1	√	KYIPGTK + bs31(1)
298	2890	+2	96	11.5	13.3	9.5	482.7704	964.5336	964.5350	-0.0014	0	√	EDLIAYLK
299	2896	+2	98	12.2	13.7	8.1	482.7705	964.5337	964.5350	-0.0012	0	√	EDLIAYLK

7. iTRAQ/TMT Quantitation Results

iTRAQ and TMT quantitation is performed in MassMatrix search engine and the quantitation results are embedded in the database search results. This manual only explains the quantitation results part of the MassMatrix search results. For the manual of MassMatrix database search results, please refer to "**MassMatrix Search Results Explained**" at

https://sourceforge.net/projects/massmatrix/files/MassMatrix_Manuals/MassMatrix%20Search%20Results%20Explained.pdf/download

Main Html



Search parameters for quantitation.

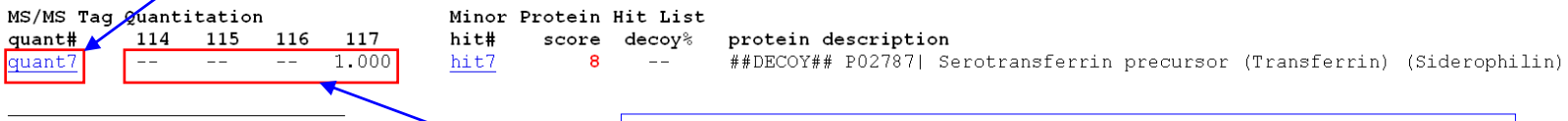
Links to the detailed statistical quantitation information about the protein matches. Click on those links to go to Protein Quantitation View for the protein matches. See "**Protein Quantitation View**" Section for more details).

A table of relative abundances of different labels for the protein matches. "--" indicates that the report ion for the label of that protein match was not observed, i.e. relative abundance ≡ 0. Σ(relative abundances) ≡ 1.0 for a protein match.

Minority Report also contains a list of protein matches of scores smaller than a critical value, and their quantitation results.

Minority Report – Protein Matches with Low Scores

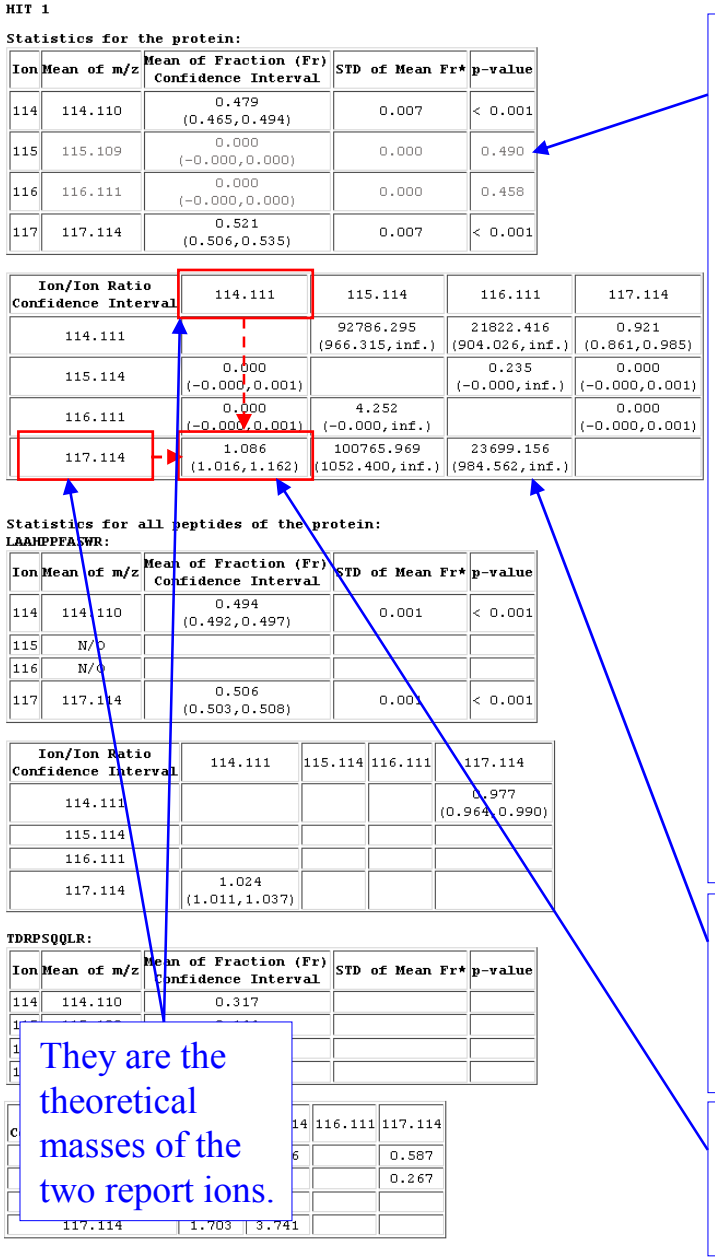
Links to the detailed statistical quantitation information about the protein matches. Click on those links to go to Protein Quantitation View for the protein matches. See "**Protein Quantitation View**" Section for more details).



MS/MS Tag Quantitation					Minor Protein Hit List			
quant#	114	115	116	117	hit#	score	decoy%	protein description
quant7	--	--	--	1.000	hit7	8	--	##DECOY## P02787| Serotransferrin precursor (Transferrin) (Siderophilin)

A table of relative abundances of different labels for the minor protein matches. "--" indicates that the report ion for the label of that protein match was not observed, i.e. relative abundance ≡ 0. Σ(relative abundances) ≡ 1.0 for a protein match.

Protein Quantitation View – Quantitation Details of A Protein Match



HIT 1

Statistics for the protein:

Ion	Mean of m/z	Mean of Fraction (Fr) Confidence Interval	STD of Mean Fr*	p-value
114	114.110	0.479 (0.465,0.494)	0.007	< 0.001
115	115.109	0.000 (-0.000,0.000)	0.000	0.490
116	116.111	0.000 (-0.000,0.000)	0.000	0.458
117	117.114	0.521 (0.506,0.535)	0.007	< 0.001

Ion/Ion Ratio Confidence Interval	114.111	115.114	116.111	117.114
114.111		92786.295 (966.315,inf.)	21822.416 (904.026,inf.)	0.921 (0.861,0.985)
115.114	0.000 (-0.000,0.001)		0.235 (-0.000,inf.)	0.000 (-0.000,0.001)
116.111	0.000 (-0.000,0.001)	4.252 (-0.000,inf.)		0.000 (-0.000,0.001)
117.114	1.086 (1.016,1.162)	100765.969 (1052.400,inf.)	23699.156 (984.562,inf.)	

Statistics for all peptides of the protein:

LAAHPPFASWR:

Ion	Mean of m/z	Mean of Fraction (Fr) Confidence Interval	STD of Mean Fr*	p-value
114	114.110	0.494 (0.492,0.497)	0.001	< 0.001
115	N/A			
116	N/A			
117	117.114	0.506 (0.503,0.508)	0.001	< 0.001

Ion/Ion Ratio Confidence Interval	114.111	115.114	116.111	117.114
114.111				0.977 (0.964,0.990)
115.114				
116.111				
117.114	1.024 (1.011,1.037)			

TDRPSQQLR:

Ion	Mean of m/z	Mean of Fraction (Fr) Confidence Interval	STD of Mean Fr*	p-value
114	114.110	0.317		

C	14	116.111	117.114
	6		0.587
			0.267
117.114	1.703	3.741	

Quantitation for the protein

Quantitation for all the peptides of protein.

They are the theoretical masses of the two report ions.

A table contains the statistics of relative abundances for all the iTRAQ or TMT labels of the protein. The statistics is obtained from the quantitation results of all the spectral peptide matches of the protein. No statistics for the protein will be available if there are less than 3 spectral matches for the protein.

Ion: Report ions of the iTRAQ or TMT labels.

Mean of m/z: Observed *m/z* values of the report ions.

Mean of Fraction (Fr): Mean values of relative abundances of the report ions.

Σ(mean of fraction) $\equiv$ 1.0.

Confidence Interval: 95% confidence intervals of relative abundances of the report ions.

STD of Mean Fr*: Standard deviation of relative abundances of the report ions.

p-value: p-values of the abundance of the report ions.

A p-value > 0.05 for a report ion indicates that there is not enough statistical evidence showing the existence of that report ion. That report ion is shown in grey.

A table contains the statistics of ratios between all the iTRAQ or TMT labels of the protein. The statistics is obtained from the quantitation results of all the spectral peptide matches of the protein.

This cell contains the ratio and its 95% confidence interval between ion 117 and ion 114, i.e. A_{117}/A_{114} and its 95% confidence interval for the protein.

Protein Quantitation View – Quantitation Details of A Protein Match (Con't)

HIT 1

Quantitation for the protein

Statistics for the protein:

Ion	Mean of m/z	Mean of Fraction (Fr) Confidence Interval	STD of Mean Fr*	p-value
114	114.110	0.479 (0.465,0.494)	0.007	< 0.001
115	115.109	0.000 (-0.000,0.000)	0.000	0.490
116	116.111	0.000 (-0.000,0.000)	0.000	0.458
117	117.114	0.521 (0.506,0.535)	0.007	< 0.001

Ion/Ion Ratio Confidence Interval	114.111	115.114	116.111	117.114
114.111		92786.295 (966.315,inf.)	21822.416 (904.026,inf.)	0.921 (0.861,0.985)
115.114	0.000 (-0.000,0.001)		0.235 (-0.000,inf.)	0.000 (-0.000,0.001)
116.111	0.000 (-0.000,0.001)	4.252 (-0.000,inf.)		0.000 (-0.000,0.001)
117.114	1.086 (1.016,1.162)	100765.969 (1052.400,inf.)	23699.156 (984.562,inf.)	

Quantitation for all the peptides of protein.

Statistics for all peptides of the protein:

LAAHPPFASWR:

Ion	Mean of m/z	Mean of Fraction (Fr) Confidence Interval	STD of Mean Fr*	p-value
114	114.110	0.494 (0.492,0.497)	0.001	< 0.001
115	N/O			
116	N/O			
117	117.114	0.506 (0.503,0.508)	0.001	< 0.001

Ion/Ion Ratio Confidence Interval	114.111	115.114	116.111	117.114
114.111				0.977 (0.964,0.990)
115.114				
116.111				
117.114	1.024 (1.011,1.037)			

TDRPSQQLR:

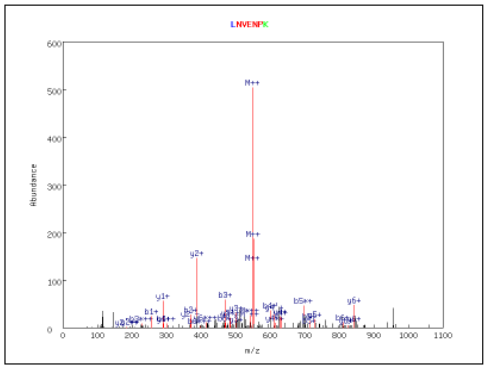
Ion	Mean of m/z	Mean of Fraction (Fr) Confidence Interval	STD of Mean Fr*	p-value
114	114.110	0.317		
115	115.109	0.144		
116	N/O			
117	117.114	0.539		

Ion/Ion Ratio Confidence Interval	114.111	115.114	116.111	117.114
114.111		2.196		0.587
115.114	0.455			0.267
116.111				
117.114	1.703	3.741		

Quantitation for all the peptides of the protein. The statistics of a peptide is obtained from the quantitation results of all the spectral matches of that peptide. No statistics for the peptide will be available if there are less than 3 spectral matches for the peptide.

All the fields for peptide statistics reports are the same as those for protein statistics report (see the previous slide for details).

Quantitation in Peptide View



#	b++	b*++	b++	b+	b*++	b+	seq	y++	y*++	y++	y+	y*++	y+	#
1			129.60			258.19	L	542.32	542.82	551.33	1083.64	1084.62	1101.65	M
2		178.11	186.62		355.21	372.24	N	413.73	414.22	422.74	826.45	827.44	844.46	6
3		227.64	236.16		454.28	471.30	V	356.71	357.20	365.71	712.41	713.40	730.42	5
4	291.67	292.16	300.68	582.34	583.32	600.35	E	307.17	307.67	316.18	613.34	614.33	631.35	4
5	348.69	349.19	357.70	696.38	697.36	714.39	N	242.65	243.15	251.66	484.30	485.28	502.31	3
6	397.22	397.71	406.23	793.43	794.42	811.44	P	185.63	186.12	194.64	370.26	371.24	388.27	2
							K	137.11	137.60	146.11	273.20	274.19	291.21	1

iTRAQ and TMT quantitation for peptide matches are also embedded in **Peptide View** of peptide matches. This section explains the quantitation results part of the Peptide View. For the search results part of the peptide view, please refer to "**MassMatrix Search Results Explained**" at

https://sourceforge.net/projects/massmatrix/files/MassMatrix_Manuals/MassMatrix%20Search%20Results%20Explained.pdf/download.

Please go to next slide for details of "Quantitation in Peptide View".

ITRAQ quantitation

Ion	Obs. m/z	Fraction	Obs. Abund.
114	114.110	0.389	22.753
115	N/O		
116	N/O		
117	117.121	0.611	35.795

Statistics for this peptide

Ion	Mean of m/z	Mean of Fraction (Fr) Confidence Interval	STD of Mean Fr*	p-value
114	114.198	0.446 (0.398,0.493)	0.021	< 0.001
115	115.250	0.002 (-0.000,0.009)	0.003	0.296
116	116.011	0.009 (0.005,0.014)	0.002	< 0.001
117	117.125	0.543 (0.499,0.588)	0.019	< 0.001

Ion/Ion Ratio Confidence Interval	114.111	115.114	116.111	117.114
114.111		265.077 (39.017,inf.)	47.210 (25.894,129.764)	0.820 (0.651,1.026)
115.114	0.004 (-0.000,0.026)		0.178 (-0.000,2.570)	0.003 (-0.000,0.020)
116.111	0.021 (0.008,0.039)	5.615 (0.389,inf.)		0.017 (0.006,0.031)
117.114	1.220 (0.975,1.536)	323.304 (49.232,inf.)	57.580 (32.672,154.042)	

*STD (standard deviation) and p-value of ion abundance are only available when the peptide has more than three matches

All possible peptide matches for this spectrum

Quantitation in Peptide View – Con't

ITRAQ quantitation

Ion	Obs. m/z	Fraction	Obs. Abund.
114	114.110	0.389	22.753
115	N/O		
116	N/O		
117	117.121	0.611	35.795

A table contains the observed absolute and relative abundances of the report ions for all the iTRAQ or TMT labels of this specific spectral peptide match.
Ion: Report ions
Obs. m/z: Observed *m/z* values of the report ions. "N/O" means "not observed".
Fraction: Relative abundances of the report ions.
Obs. Abund.: Observed absolute abundances of the report ions.

Statistics for this peptide

Ion	Mean of m/z	Mean of Fraction (Fr) Confidence Interval	STD of Mean Fr*	p-value
114	114.198	0.446 (0.398,0.493)	0.021	< 0.001
115	115.250	0.002 (-0.000,0.009)	0.003	0.296
116	116.011	0.009 (0.005,0.014)	0.002	< 0.001
117	117.125	0.543 (0.499,0.588)	0.019	< 0.001

Statistics results of the peptide for the spectral match. These results are obtained from the quantitation results of this match and all other matches for this peptide. No statistics for this peptide will be available if there are less than 3 spectral matches for the peptide.

All the fields for peptide statistics reports are the same as those for protein statistics report.

Ion/Ion Ratio Confidence Interval	114.111	115.114	116.111	117.114
114.111		265.077 (39.017,inf.)	47.210 (25.894,129.764)	0.820 (0.651,1.026)
115.114	0.004 (-0.000,0.026)		0.178 (-0.000,2.570)	0.003 (-0.000,0.020)
116.111	0.021 (0.008,0.039)	5.615 (0.389,inf.)		0.017 (0.006,0.031)
117.114	1.220 (0.975,1.536)	323.304 (49.232,inf.)	57.580 (32.672,154.042)	

*STD (standard deviation) and p-value of ion abundance are only a

8. Quantitation Using SILAC/^{15}N Labeling

1. Overview

MassMatrix online server is a database search engine that can be used for both database search and quantitation analysis of LC-MS/MS data with SILAC or ^{15}N labeling. The quantitation analysis is performed via post-hoc mode by a stand-alone program written in Python.

2. Pre-processing of your RAW data files

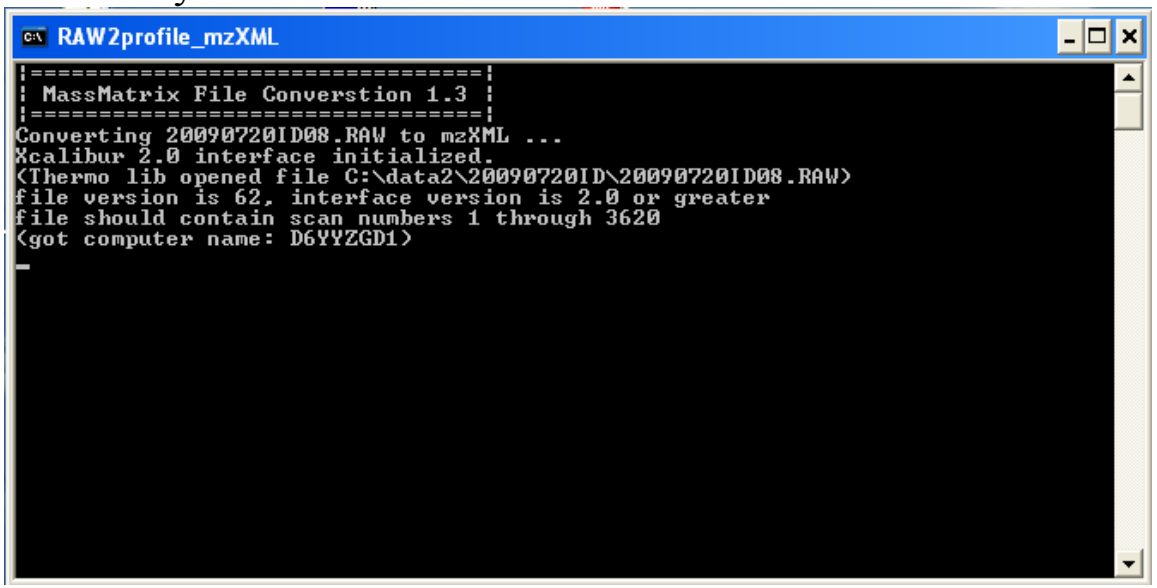
RAW data files from mass spectrometers cannot be directly searched in MassMatrix. You need to convert RAW data files to a proper format. For quantitation via SILAC or ^{15}N labeling, only mzXML files in a profile format are accepted. Other data formats, such as MGF and centroided mzXML, can be used only for database search and quantitation via iTRAQ and Tandem Mass Tag in MassMatrix, but not for quantitation analysis via SILAC and ^{15}N labeling.

To convert RAW data files to profile mzXML files, please go to

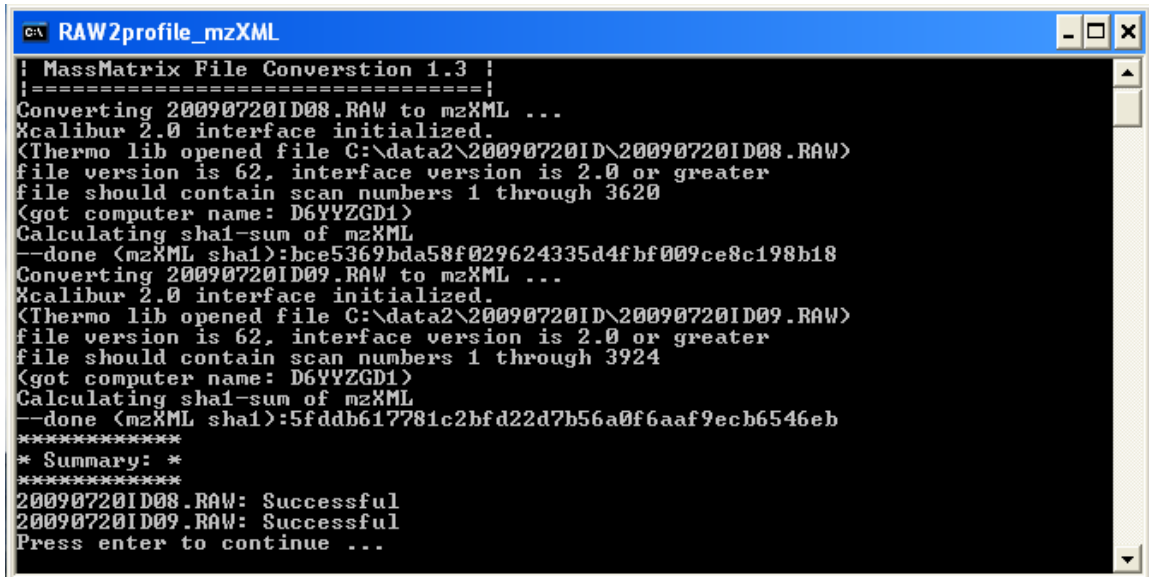
https://sourceforge.net/projects/massmatrix/files/MM_File_Conversion.zip/download

to download a software package, called MM File Conversion Tools. Install the package on your computer. After installation, there will be several programs on your desktop. You should use the one named “RAW2profile_mzXML”. If you installed an early version of the software and it does not have “RAW2profile_mzXML”, please download the latest version of the software via the above link and install it.

Grab all the RAW files that you want to convert using mouse and move them to the "RAW2profile_mzXML" program. A DOS window will pop up and the files will be converted to profile mzXML files one by one.



After the conversion is finished, the program will give you a summary report. Check the summary report to make sure that all the files are successfully converted. If any of them are not successful, you may want to redo those that are not successfully converted.

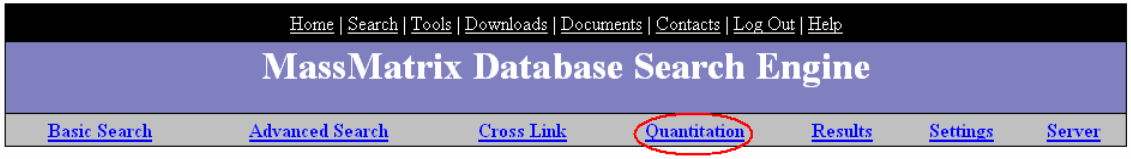


3. Database search and quantitation analysis

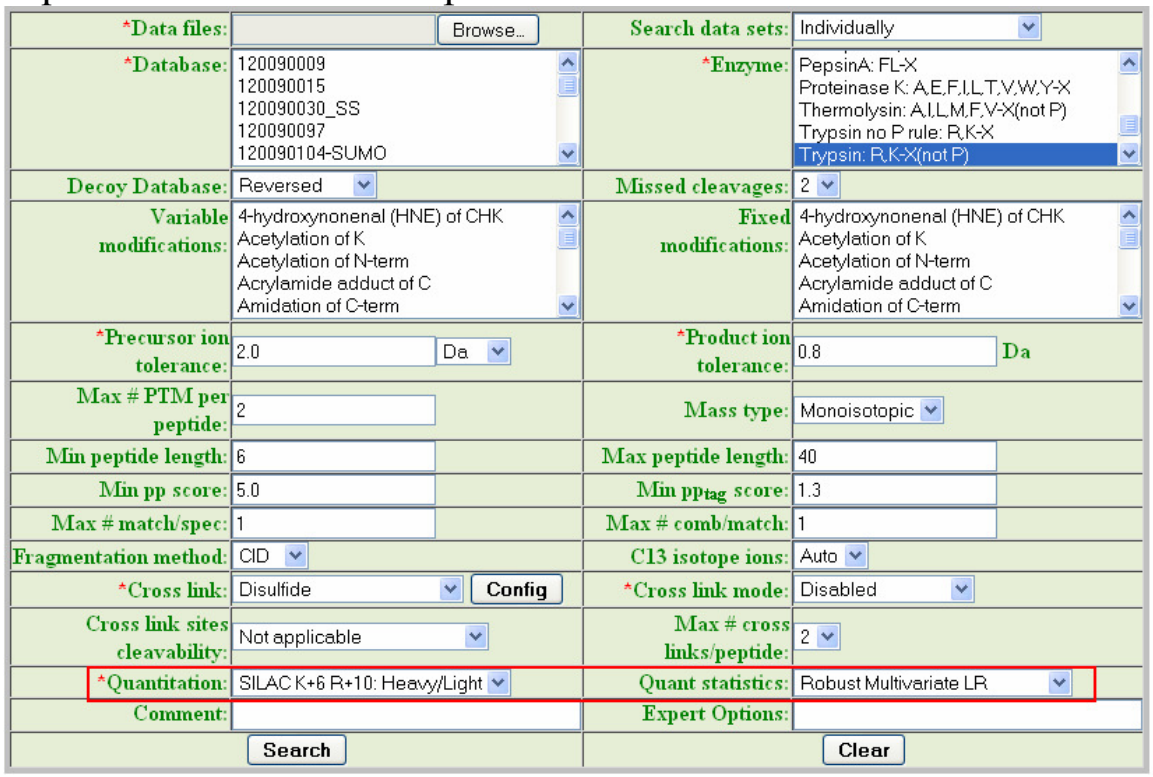
Please go to a MassMatrix online server and click on “Log In” to log in. You will need an account to log in the server to do searches. If you don’t have an account, please email the administrator of the server to request a new account. You may also log in as a guest.

After logging, please click on the “Search” tab at the top to go to the search engine.

Then click on the “Quantitation” tab to go to the quantitation function of the search engine.



A search form for quantitation will show up as follows



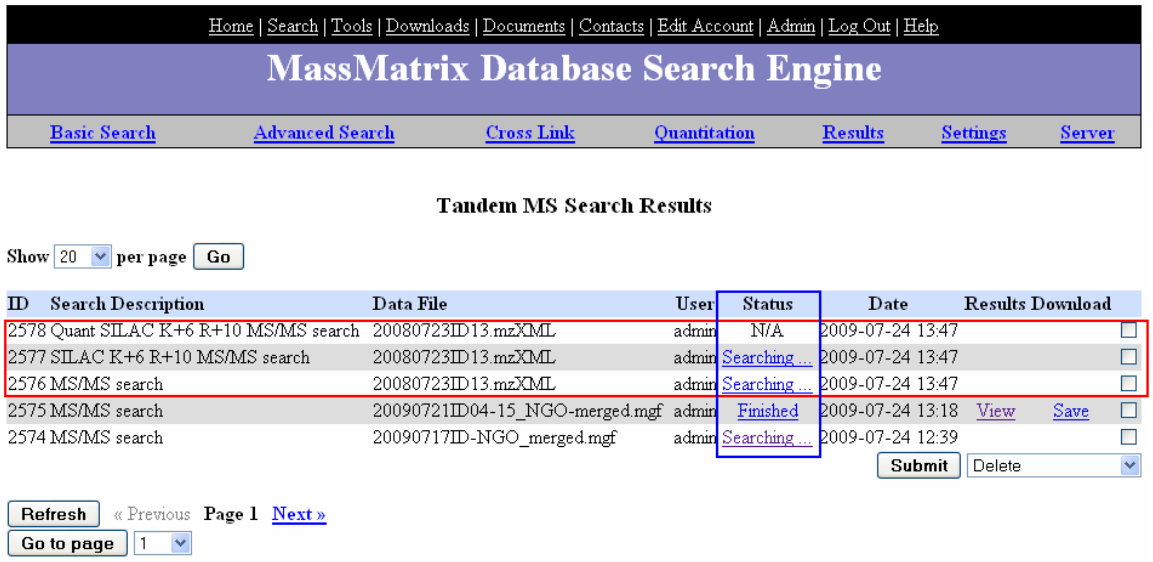
Please upload your mzXML data files and fill out the search form. You may upload multiple data files and search them at once as long as they share the same set of parameters. Most of the parameters are for the database search only. For the parameter settings in MassMatrix, please refer to the online help file. Only two parameters are for the quantitation as highlighted in the above figure. The first one is the SILAC method that you used. You may configure a new SILAC method if the one you use is not in the list. Please refer to the online help file at

https://sourceforge.net/projects/massmatrix/files/MassMatrix_Manuals/MassMatrix%20Server%20Settings.pdf/download
for more info. The second parameter is the statistical method for quantitation. Details of those methods are not covered in this help file. But the mathematical proofs and also the evaluation of those different methods will be published in a scientific journal. It is recommended that you always use the default method.

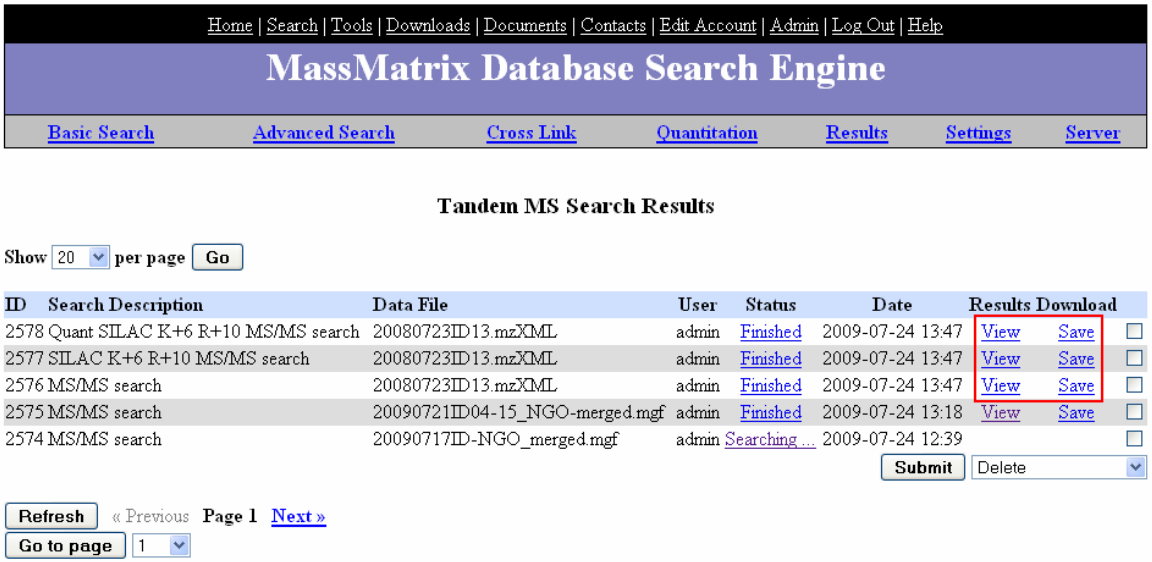
After filling out the form, click "Search" to submit the search. There will be two database searches and one quantitation analysis will be submitted to the server. The two database searches are the searches for proteins with light and heavy labeling respectively. The quantitation analysis is performed by a post-hoc quantitation analysis module written in Python. The quantitation module takes the search results from the two database searches and performes the quantitation analysis for you. Therefore, the two database searches will be processed by the server first. After the two searches for your job are done, the quantitation analysis for your job will be processed.

Please go to the result page to view all your submitted jobs by clicking on the "Results" tab.

You may also check the status of your jobs by clicking on their status links.



After the searches and quantitation are done, you can view and download the search results and quantitation results.



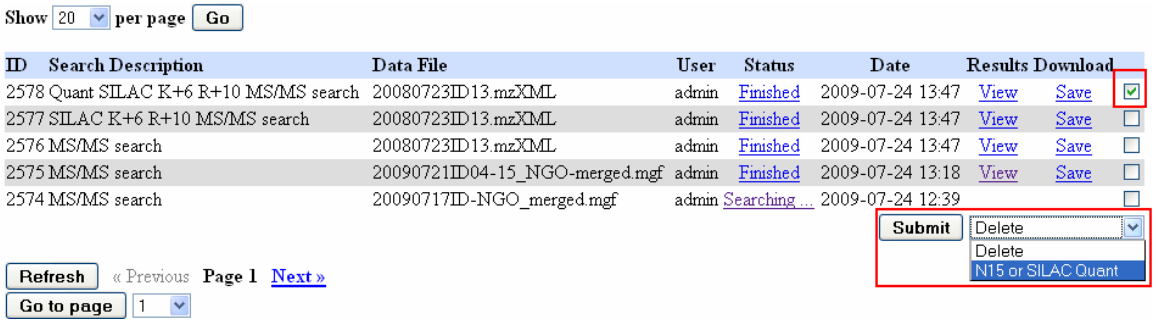
The quantitation results can be downloaded as a zip file. After downloading the zip file, please unzip the file. There are three txt files: a parameter setting file used during quantitation, a file containing all peptides with quantitation information, and a file containing all proteins with quantitation information. Please open the files containing peptides and proteins with quantitation information in excel. Abbreviations used in the result files are listed in the following table.

Abbreviation	Description
STD	Standard Deviation
LB of 95% CI	Lower bound of 95% confidence interval

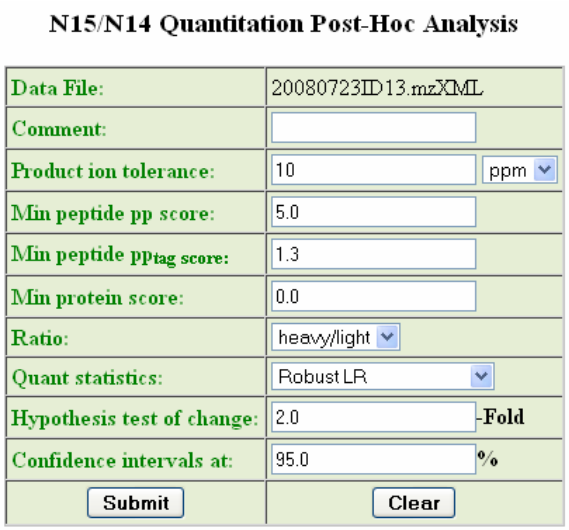
UB of 95% CI	Upper bound of 95% confidence interval
P(change>=2.0-fold)	Probability that the change > 2.0 fold
P(sig. change)	Probability that there is a significant change

4. Post-hoc quantitation analysis

The online server allows you redo the post-hoc quantitation without submitting or redoing the database searches. Go to the search result page and locate the quantitation job that you want to do the post-hoc quantitation analysis. Select the job and choose "N15 or SILAC Quant" in the submit option and click "Submit".



A form for post-hoc quantitation will be shown.



Please fill out the quantitation post-hoc analysis form and click "Submit" to redo your quantitation. This post-hoc analysis allows you to set up a threshold for protein matches so that only those with scores bigger than the threshold will be done for quantitation. In this way, false protein identifications can be controlled. Also, you may change the critical value for the hypothesis testing during quantitation. By default, all the ratios calculated for protein matches will be tested against a critical value of 2.0.

After the post-hoc quantitation is submitted, the job will be in the result list and can be viewed by going to the result page.

Appendix: Chemical Formula Syntax

Chemical Formula Syntax

Rule 1: Normal chemical formulas for chemical compounds are supported.

Methane: CH4 (or CHHHH if you prefer)

Water: H2O or HHO

Rule 2: parentheses are supported for repeating units in the formula.

Glucose: (CH2O)6 equivalent to C6H12O6

Rule 3: Nested parentheses ARE NOT SUPPORTED.

CH3((CH2CHOH)2O)5CH3 *IS NOT ACCEPTABLE*.

Rule 4: Negative numbers are supported.

CH5H-1 equivalent to CH4.

Rule 5: amino acid residues (3-letter abbr. or 1-letter abbr.) are supported, but HAVE TO BE SURROUNDED BY CURLY BRACKETS.

Glycine residue: {G} = {Gly} = C2H3NO

Aspartic acid residue: {Asp} = {D} = C4H5NO3

Glycine amino acid: H{G}OH = H{Gly}OH = C2H5NO2

Aspartic acid: H{Asp}OH = H{D}OH = C4H7NO4

Chemical Formula Syntax

Rule 6: "{amino acid sequence}" cannot be nested in a "()" for repeated sequence. But "()" can be used inside "{}".

A peptide with a sequence of GGAEDGGAED: H{GGAEDGGAED}OH = H{(GGAED)2}OH

NOTE: H({GGAED})2OH IS NOT ACCEPTABLE.

NOTE: {G2} IS NOT ACCEPTABLE EITHER. {(G)2} is acceptable and equal to {GG}.

Rule 7: Only the following isotopes for Hydrogen, Oxygen, Nitrogen and Carbon are accepted.

2H: D

3H: T

13C: C(13)

18O: O(18)

15N: N(15)

Lysine labeled with six 2H: C6H6D6N2O

Arginine labeled with six 13C and four 15N: C(13)6H12N(15)4O

Contacts

http://www.massmatrix.org
http://www.massmatrix.net

For more information about the MassMatrix search engine and results, please contact

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